APPLICATIONS OF REVERSE MICELLES IN NORMAL PHASE LIQUID CHROMATOGRAPHY

BY

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TO JILL:

Who has provided an immeasurable amount
of support and understanding throughout
the years.

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Reverse micelles which are formed by certain surfactants in nonpolar solvents have potential as strength modifiers, for gradient elution and as water scavengers in normal phase liquid chromatography (NPLC). In this work, Aerosol OT, a surfactant which exhibits classic micelle forming behavior in nonpolar solvents was used to produce the reverse micelles. The adsorption behavior of Aerosol OT on stationary phase materials used in NPLC and the effect of surfactant concentration on solute retention was investigated for several selected chromatographic systems. The results show that reverse micellar NPLC systems behaved

predictably when experimental parameters such as solute type, stationary phase, water, polar solvent modifier and temperature were varied.

Adsorption isotherms were characterized by strong adsorption behavior and saturation of available sites occurred at the lowest concentrations tested.

The effect of surfactant concentration on solute retention showed a clear cut critical micelle concentration behavior by Aerosol OT in mobile phase solvents which included large amounts of water and polar alcohol modifier. Consistent with pseudophase chromatographic theory, as surfactant concentration was increased above the critical micelle concentration, solute retention decreased in direct proportion. Binding constants and critical micelle concentrations were determined in these chromatographic systems.

Several experiments were conducted which illustrated the ability of Aerosol OT reverse micelles to mask the presence of large amounts of water in NPLC systems. The reverse micelles act by encapsulating the water and by adsorbing onto a water layer on the stationary phase.

Reverse micelles provide a relatively strong mobile phase modifier and this combined with the observed flatness of the adsorption isotherms accommodates gradient elution. A gradient

chromatographic separation was demonstrated and showed that system reequilibration was rapid, although the strong adsorption of Aerosol OT in the ultraviolet region is a limitation.

The presence of Aerosol OT in the chromatographic system provided a positive effect on efficiency and band symmetry at low surfactant concentrations but at higher surfactant concentrations, efficiency was lowered due to increased solution viscosity.

CHAPTER 1

INTRODUCTION

Normal Phase Liquid Chromatography

Around the year 1900, Michael S. Tswett utilized and theoretically described the technique of differential adsorption of components in a mixture onto a solid sorbent to achieve their separation (Ettre, 1971). Thus, liquid-solid or adsorption chromatography was the technique used in what is considered to be the birth of chromatography. Adsorption chromatography continued to be the sole chromatographic technique in use until the early 1940s, when Martin and Synge invented partition chromatography; a technique in which one liquid is passed over a second immiscible liquid.

Liquid-liquid partition chromatography could be operated in either the normal phase mode, with the more polar solvent coated on the stationary support and the nonpolar solvent as the eluent, or in the reversed phase mode with the solvent polarities switched. Inherent problems with the liquid-liquid technique soon led to phases which were chemically bonded onto the stationary support.

Modern normal phase liquid chromatography (NPLC) encompasses both the traditional liquid-solid chromatography as well as chromatography in the normal phase mode on bonded phases. The retention mechanism in both techniques is primarily adsorption (Snyder, 1983).

Snyder has provided the theoretical foundation for a quantitative treatment in his book on adsorption chromatography (Snyder, 1968). More recently, NPLC has been the subject of several reviews (Saunders, 1977; Abbott, 1980; Snyder, 1983a; Engelhardt and Elgass, 1980; Snyder, 1983b).

Liquid chromatography is primarily applicable to analysis of organic compounds, especially those that are nonvolatile or too thermally labile to be analyzed by gas chromatography. Low volatility of solutes occurs because of large molecular size, hydrogen bonding polar groups or ionic character. Typically, ionic solutes can be analyzed by ion exchange chromatography and very large solutes are best separated by size exclusion chromatography. This leaves a general category of organic analytes which are of small to intermediate size and generally contain polar functional groups which can be analyzed by either reversed phase liquid chromatography (RPLC) or NPLC. Areas of application include environmental,

pharmaceutical, polymer, biomedical, and industrial process control fields.

NPLC has a number of advantages and areas of application with regard to RPLC (Abbott, 1980);

a) NPLC separations are primarily due to the polar functional groups of a solute and the hydrocarbon portion of the molecule contributes very little to retention. Consequently class separations can be obtained by NPLC.

b) RPLC separations are largely due to the hydrophobic nature and NPLC separations based on the hydrophilic portion will provide a radically different selectivity.

c) Because the physical process of adsorption is dependent on molecular topography, NPLC is especially useful for isomeric separations.

d) RPLC uses aqueous/hydro-organic mobile phases. Solutes which are insoluble or unstable in these solvents can be analyzed by NPLC.

e) Highly polar organic compounds are often unretained in RPLC and can be separated by NPLC. In fact NPLC is applicable to a wide range of solute polarities.

f) A large number of different solvents can be used in NPLC to maximize the chemical selectivity of the separation. In addition, the available stationary phase materials provide a wide range of chemical selectivities.

g) The lower viscosities of solvents used in NPLC allow lower operating pressures or higher flow rates. The increased volatility of these solvents compared to aqueous mobile phases facilitates solvent removal for subsequent solute recovery or analysis using detectors such as mass spectrometers.

Currently NPLC accounts for approximately 20 percent of all high performance liquid chromatography (HPLC) split about evenly between the liquid-solid and

Table 1.1 HPLC analytical usage mode, survey-1987.

Chromatographic Mode	Percentage of use
Reversed Phase	56.9
Normal Phase, total	17.1
Normal Bonded Phase	9.0
Normal Liquid-Solid	8.1
Ion Exchange	16.6
Size Exclusion	5.7
Chiral	1.5
Hydrophobic Interaction	2.5

Data adapted from (Majors, 1988).

bonded phase techniques as shown in Table 1.1 (Majors, 1988). RPLC accounts for about 60 percent of all HPLC. The preference for RPLC can be attributed to several factors. RPLC is a popular technique and most HPLC users are familiar with it and it's capabilities. Lifescience applications are prevalent and often lend themselves more to RPLC. In addition, NPLC tends to have some performance problems which limit it's utility. However, the advent of bonded phases has minimized these problems and there is a general feeling that many current RPLC separations would be more properly done by NPLC if there was a better understanding of it's capabilities (Snyder, 1983b; Abbott, 1980).

The normal phase mode is used extensively in thin layer and preparative column chromatography. However, these applications are not as demanding in terms of efficiency and reproducibility as HPLC. Many of the problems with the NPLC technique are caused by the presence of high energy sites on the adsorption surfaces and the large distribution in the energy levels of the adsorption sites.

The high energy adsorption sites cause strong retention of ionic and highly polar solutes. Not all of the sites retain the solute equally and slow

desorption from high energy sites causes tailing of chromatographic bands with these solute types.

Water is also strongly retained by these high energy sites and ubiquitous trace levels of moisture in chromatographic solvents are removed by the stationary phase during the course of analysis. This water builds up on the adsorbent and deactivates the sites resulting in decreasing retention of the solute as analysis proceeds. For chromatography on nonbonded stationary phases, thorough drying of the solvents or addition of a large constant amount of water to the mobile phase to saturate these sites is usually recommended (Snyder, 1968; Saunders, 1977; Abbott, 1980; Engelhardt and Elgass, 1980).

These high energy adsorption sites also cause problems with gradient elution NPLC. In gradient elution chromatography, the concentration of the strong solvent is varied to increase eluent strength. This allows the simultaneous analysis of weakly and strongly retained solutes. As the concentration of the strong solvent is increased, the high energy adsorption sites preferentially remove it from the mobile phase in a process known as solvent demixing. This results in a weaker mobile phase than predicted. The high energy sites also cause a problem when the solvent is returned to its original composition prior to the next

analytical run. Slow desorption of the strong solvent from these sites requires long reequilibration times which increases analysis time.

The basic premise of Snyder's theoretical treatment of adsorption chromatography is that a displacement competition occurs between the solute and solvent molecules on the adsorption surface (Snyder, 1968). Initially the stationary phase is covered by an adsorbed monolayer of solvent molecules, and as the solute molecule moves down the column, it displaces one or more solvent molecules (depending on the relative molecular sizes). Subsequently the solute is displaced from the surface by additional solvent molecules and continues down the column. Solute retention is dependent on the relative adsorption energies of the solute (E_x) and solvent (E_s) molecules. If the assumption is made that solute-solvent and solvent-solvent interactions in the mobile phase cancel each other, then the net adsorption energy for a solute displacing n solvent molecules is

$$E = E_x - nE_s \qquad \text{(eqn. 1.1)}$$

This net adsorption energy is approximately equal to the logarithm of the capacity factor (log k).

Snyder (1968) has described a solvent strength parameter, e^o, which corrects the solvent adsorption

energy for the area required by an adsorbed solvent molecule (A_s):

$$e^\circ = E_s/A_s \qquad \text{(eqn. 1.2)}$$

Since n is the ratio of the molecular areas of the solute (A_x) and the solvent, ($n = A_x/A_s$), equation 1.2 can be written as

$$\log k = E_x - A_x e^\circ \qquad \text{(eqn. 1.3)}$$

According to this equation, a solutes retention depends on it's adsorption energy and adsorption area. The adsorption energy of a molecule can be calculated by summing the adsorption energies of the various organic functional groups which comprise it. Solute molecular areas can be obtained by summing the areas of the individual atoms in the molecule. Group adsorption energies and atomic areas have been tabulated by Snyder (1968).

According to equation 1.3, the solvent strength can be varied to alter solute retention. Table 1.2 lists e° values for commonly used chromatographic solvents. In NPLC, a low e° solvent such as an alkane or a chlorofluoroalkane is used as the base solvent and a polar modifier solvent is added to adjust the mobile phase strength. The strength of binary solvent mixtures

Table 1.2 Strengths of chromatographic solvents on alumina.

Solvent	e°
1,1,2 trichlorotrifluoroethane	-0.25
isooctane	0.01
n-hexane	0.01
n-pentane	0.01
carbon disulfide	0.15
carbon tetrachloride	0.18
isopropyl chloride	0.29
toluene	0.29
ethyl ether	0.38
n-octanol	0.5
methylene chloride	0.42
n-butanol	0.7
isopropanol	0.82
ethyl acetate	0.58
chloroform	0.40
methylethylketone	0.51
nitroethane	0.6
acetone	0.56
dioxane	0.56
aniline	0.62
dimethylsulfoxide	0.75
ethanol	0.88
acetonitrile	0.65
methanol	0.95
ethylene glycol	1.11

Data adapted from Snyder and Kirkland (1979).

has a logarithmic dependence on the amount of the more polar solvent and can be calculated by

$$e^{\circ}_{mix} = e^{\circ}_{A} + \frac{\log(X_B \, 10^{A_B(e^{\circ}_B - e^{\circ}_A)} + X_A}{X_B} \qquad \text{(eqn. 1.4)}$$

where e°_A and e°_B denote the strength of the pure weak and strong solvents respectively, X_A and X_B are the mole fractions of the two solvents and A_B is the molecular size of the strong solvent molecule. Figure 1.1 shows the e° values for some chromatographically useful binary solvent mixtures. Solvent mixtures with the same e° values have comparable elution strengths. Consequently, mixtures using solvents from different solvent groups can be used to optimize the selectivity of a separation while keeping retention constant.

The above theory, and equation 1.3, are reasonably accurate for systems comprised of nonpolar solvents and solutes such as hydrocarbons, aromatics and chlorinated hydrocarbons. The theory is less appropriate for systems which contain more polar solvents and solutes having functional groups which preferentially interact with specific sites on the adsorbent surface (Snyder, 1983a).

Even though equation 1.3 is not completely appropriate or accurate for more polar systems, it is

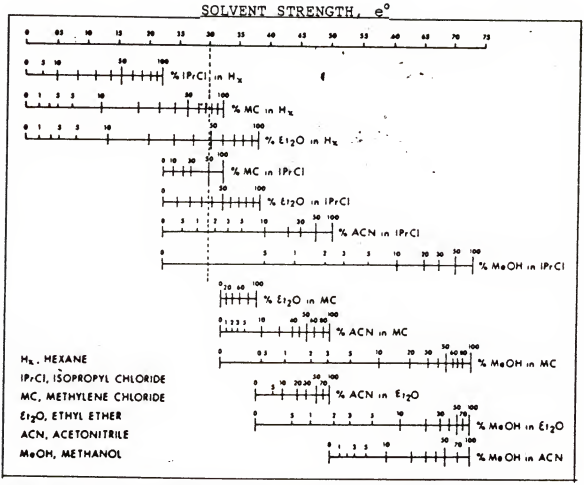


Figure 1.1 Strength of Binary Solvent Mixtures on Silica. Adapted from Saunders, 1974.

still useful in a semi-quantitative sense and is commonly used. Expressions which adjust for localization on specific adsorption sites and hydrogen bonding interactions between the solute and solvent have been developed but usually require empirical data from the specific system (Snyder, 1983a).

Important solvent parameters for liquid chromatography are solvent strength, viscosity, ultraviolet cutoff wavelength, vapor pressure, density and solvent group. Solvent group is a classification scheme for solvents according to propensity for hydrogen bonding donation or acceptance, or for dipole interaction (Snyder, 1974; Snyder, 1978).

Table 1.3 lists the stationary phase materials commonly used in NPLC. Important properties of stationary phase packings are particle diameter, particle shape, particle size distribution, pore size, surface area, adsorbent activity function and chemical selectivity of the adsorption surface. Typically NPLC packings are 5 to 10 micrometer (μm) spherical particles with 5 to 10 nanometer (nm) pores and a surface area of 200 to 500 m^2/g (Snyder and Kirkland, 1979; Majors, 1980). Activity function, α, is a measure of the adsorption strength of an adsorbent using activated alumina as a standard. Equation 1.3

Table 1.3 Common stationary phase materials for NPLC.

Packing Material	Phase/Adsorption Sites
Alumina	$=Al-O^-$ $=Al-O-H$ *
Silica	$=Si-O^-$ $=Si-O-H$
Cyano	$=Si-O-H$ $=Si-O-(CH_3)_2Si-(CH_2)_3-CN$
Amino	$=Si-O-(CH_3)_2Si-(CH_2)_3-NH_3$
Diol	$=Si-O-(CH_3)_2Si-(CH_2)_3-O-CH(OH)-CH_2(OH)$

* For the case of alumina, surface oxides and hydroxyls may provide adsorption sites, but the primary adsorption sites are cationic centers (aluminum atoms or lattice defects) buried in the lattice (Snyder, 1983a).

can be rewritten to take into account adsorption activity function

$$\log k = E_x - \alpha A_x e^{\circ} \qquad \text{(eqn. 1.5)}$$

Other adsorbents which are weaker than alumina have smaller activity functions. Water can be added to an adsorbent to deactivate the adsorption sites and decrease the activity function.

Silica is the most widely used solid adsorbent and its silanol adsorption sites are acidic in nature. Alumina, another useful adsorbent, contains basic adsorption sites in addition to surface hydroxyls and although retention for most solutes is similar to that for silica, acidic solutes are more strongly retained. Silica and alumina from which water has been removed by heating have an activity function of 1. They can be deactivated by water addition to obtain activities of around 0.5 (Snyder, 1968).

Currently available bonded phase packings include cyano, amino and diol functionalities (usually attached with a propyl chain) chemically bonded to silica particles. Diol columns have the highest activity of these materials and have a chemical selectivity similar to silica since the primary adsorption sites in both are hydroxyls (Snyder, 1983b).

Amino columns are basic in terms of chemical selectivity because the primary adsorption sites are the amino groups (Snyder and Schunk, 1982). Amino bonded phases are more retentive towards acidic solutes than silica. A problem with these packings is reactivity with solvents or solutes containing aldehyde or ketone functional groups.

The primary adsorption sites on cyano bonded phases are silanols and not the cyano groups (Weiser, et al., 1984). The cyanopropyl bonded phase restricts access to these silanol sites resulting in a weak stationary phase (α = 0.2). Only when using strong mobile phase solvents, such as alcohols, which block the silanol sites do the cyano groups become the primary adsorption sites (Weiser et al., 1984; Cooper and Smith, 1986; Suffolk and Gilpin, 1986).

In general the bonded stationary phases are less retentive than the solid adsorbents but they provide a more homogeneous adsorption surface (Snyder, 1983a). The bonded phase reduces high energy adsorption sites, consequently reducing chemisorption of water and polar solutes, and improving chromatographic performance (Weiser et al., 1984; Majors, 1980).

Reverse Micelles

It was recognized as early as 1910 by McBain that reversible association of ions to form micelles occurred in aqueous solution, but it was the late 1930's before it was realized that aggregation could also occur in nonpolar solvents (Stenus, 1984). Work on aggregation in apolar solvents remained disorganized and isolated until Singleterry published a report in 1955 which listed surfactants that formed aggregates in nonpolar solvents (Singleterry, 1955). Recently there has been increasing interest in reverse micelles and several reviews have been published (Fendler, 1976; Kitahara, 1980; Eicke, 1980; Fendler, 1982) along with the proceedings of a conference on reverse micelles (Luisi and Straub, 1984). The current areas of investigation for reverse micelles include membrane mimetics, drug delivery, petroleum recovery, detergents, lubrication, solubilization into organic solvents, catalysis, preparation of colloids, liquid-liquid extraction and artificial photosynthesis (Fendler, 1984a; Langevin, 1984).

Surfactants are substances which contain both a hydrophobic and a hydrophilic section in the same molecule. The hydrophobic portion is composed of a hydrocarbon or fluorocarbon chain of between eight and twenty carbons in length. The hydrophilic part can be

anionic, cationic, zwitterionic or contain a neutral polar group and is usually small in size compared to the hydrophobic section. Table 1.4 lists some examples of these surfactant types.

The dual nature of these molecules causes them to concentrate at interfaces and consequently they can have a large effect on solution properties even when present at low concentrations. Another important property of surfactants is the ability to form various types of aggregates in solution. Aggregates which are formed by surfactants in aqueous media include micelles, microemulsions, black lipid membranes and vesicles (Fendler, 1980; Fendler, 1984b).

Micelles in apolar solvents have an inverted structure compared to aqueous micelles and are known as reverse micelles. The energetics of formation of these aggregates in nonpolar solvents are quite different from those for normal micelles and researchers in the field have slowly come to the realization that new models for aggregation are required (Fendler, 1982).

The forces which drive micelle formation in aqueous solution are a combination of hydrophobic interactions and electrostatic repulsions. Association of surfactants in nonpolar solvents is primarily a result of dipole-dipole and ion pair interactions of the polar head groups (Fendler, 1982). The presence of

Table 1.4 Examples of common surfactant types.

Surfactant Type	Example name	Structure*
Anionic	sulfate	$R\text{-}SO_4^-\ Na^+$
	carboxylate	$R\text{-}COO^-\ Na^+$
Cationic	ammonium	$R\text{-}N^+(CH_3)_3\ Cl^-$
Nonionic	polyglycol ether	$R\text{-}(OCH_2CH_3)_6OH$
Zwitterionic	ammonium propyl-sulfate	$R\text{-}N^+(CH_2)_2\text{-}(CH_2)_3\text{-}SO_3^-$

* R is a chain of 12 to 20 carbon atoms in length.

small amounts of water is considered essential for aggregate formation (Eicke and Christen, 1978; Rouviere et al., 1979; Fendler, 1982). The observed stability of these aggregates over the large temperature range of -85° to 95°C indicates stabilization by a hydrogen bond network of water molecules interacting with the polar head groups of the surfactant (Zulauf and Eicke, 1979; Fendler, 1982).

Classical micelle formation behavior as exhibited by surfactants in aqueous systems is characterized by a reversible equilibrium between the monomers (S_1) and the aggregate of a definite size ($S_{\tilde{n}}^-$), where $\tilde{n}$ is the aggregation number

$$\tilde{n}S_1 \longrightarrow S_{\tilde{n}}^- \qquad \text{(eqn. 1.6)}$$

The aggregation numbers are large (typically $\tilde{n}$ = 50-100) in aqueous solution and monodisperse (i.e.; there is a small distribution range of micelle sizes).

Surfactants in nonaqueous solvents exhibit a range of aggregation behaviors which depend on the surfactant type and the polarity of the solvent. These behaviors have been classified into two types which can be considered opposite limiting cases (Muller, 1978). Dodecylammonium propionate (DAP) in benzene, a cationic surfactant, is considered a model for Type I behavior. It's aggregation number is small ($\tilde{n}$ = 3 to 7) and as

surfactant concentration is increased, the aggregation number continues to increase without reaching a limiting value. These aggregates are relatively polydisperse and Type I behavior is a result of sequential indefinite self-association:

$$S_1 + S_1 \longrightarrow S_2$$
$$S_2 + S_1 \longrightarrow S_3$$
$$\cdots\cdots\cdots\cdots\cdots$$
$$S_{n-1} + S_1 \longrightarrow S_{\bar{n}} \qquad \text{(eqn. 1.7)}$$

Type II behavior is typified by larger aggregation numbers ($\bar{n}$ = 12 to 30) and aggregates which reach a constant limiting size. The aggregates are primarily monodisperse and they have a fairly well defined critical micelle concentration (CMC) break when various solution properties are monitored as a function of surfactant concentration (Fendler, 1982).

Aerosol OT (AOT, sodium bis-2-ethylhexylsulfosuccinate), an anionic surfactant, is a model compound for Type II aggregation. However, aggregation is more complicated in nonpolar solvents than the monomer-micelle equilibria observed in aqueous media. Figure 1.2 shows an aggregation scheme proposed by Gelade et al. (1986) for reverse micelles. This model features the formation of linear premicellar aggregates

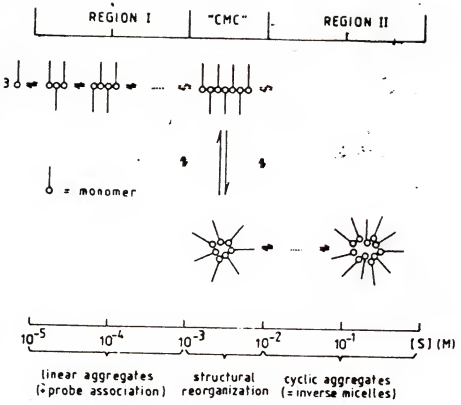


Figure 1.2 Aggregation Scheme for Surfactants in nonpolar solvents featuring linear premicellar aggregates and reorganization to a closed structure at the CMC. Adapted from Gelade, et al., 1986.

(LPMAs), the presence of which has been postulated by several workers (Eicke and Christen, 1978; Eicke et al., 1975; Tamura and Schelly, 1979; Tamura and Schelly, 1981). Evidence suggests that the principal LPMAs are dimers, trimers and hexamers. LPMAs begin to form at surfactant concentrations as low as 10^{-5} M. Association of probe molecules with LPMAs and solubilization at surfactant concentrations below the CMC have been observed (Eicke, 1980; Gelade et al., 1986).

The CMC break in solution properties, which is exhibited by Type II surfactants, may be the result of a reorganization of linear aggregates to a closed micelle structure. Since this behavior is quite different from the monomer-micelle equilibria in aqueous systems, and because the surfactant concentration at which it occurs is dependent on solvent polarity and the presence of other additives such as water, these regions of change in solution properties are generally considered to be "operational" CMC's. They occur at surfactant concentrations of approximately 10^{-3} M and are a result of the formation of hydrogen bond networks with the small amounts of water always present in these systems (Eicke, 1980). In general, the sharpness of the CMC break is predicted to increase as the aggregation number increases.

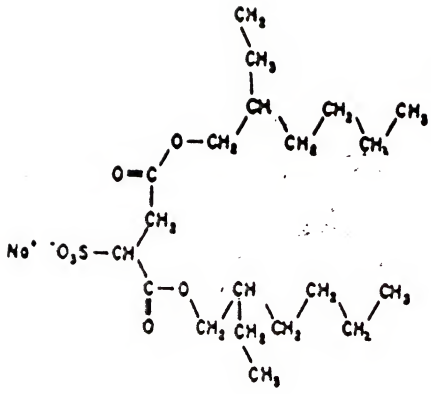


Figure 1.3 Molecular Structure of Aerosol OT. Adapted from Wong et al. 1977.

Aerosol OT is the most widely studied surfactant that forms reverse micelles. It also forms normal micelles in aqueous systems and is one of the few surfactants to form three component (oil, water, and surfactant) microemulsions at room temperature (Chen et al., 1986). The molecular structure of the AOT molecule is shown in Figure 1.3. It is a top-shaped molecule with a molecular length of 120 nm, a molecular area of 5,500 nm^2 and a molecular weight of 444.45 (Zulauf and Eicke, 1979).

Aerosol OT forms reverse micelles which are monodisperse and spherical or cylindrical in shape. The aggregation number depends on the polarity of the solvent and it's hydrogen bonding ability. Thus AOT is monomeric (no micelle formation) in methanol, while $\bar{n}$ = 6 in t-amyl alcohol, 17 in carbon tetrachloride, and 25 to 30 in alkane solvents (Peri, 1969). Hydrogen bonding solvents severely curtail aggregation and even one percent of methanol in toluene has been shown to inhibit micelle formation (Fryar and Kaufman, 1969). Solvents also cause shifts in the CMC; in general, the more nonpolar the solvent, the lower the CMC. These solvent effects on aggregation and CMC are predictable. Less polar solvents force the surfactant molecules to associate with each other while polar, and in

particular, hydrogen bonding solvents will associate with the surfactant molecules and hinder aggregation.

Table 1.5 lists literature aggregation numbers for Aerosol OT in various solvents as determined by several different techniques. There is a certain amount of discrepancy in the values obtained by the different techniques and among the different workers but several tendencies are apparent. The aggregation number increases with decreasing solvent polarity and is independent of temperature. It has also been found to be almost completely independent of surfactant concentration.

Table 1.6 lists literature CMC values for Aerosol OT. CMC increases slightly as the temperature is increased (Ueno and Kishimoto, 1977; Jean et al., 1979) and is expected to decrease as the solvent becomes more nonpolar. The presence of water and other polar compounds which help stabilize the aggregate can shift the CMC to lower values (Eicke et al., 1975).

Another important aspect of surfactant behavior in solution is the ability to dramatically increase the solubility of many substances in a given solvent. This is a result of encapsulation of solutes which are incompatible with the solvent into the interior of the micelle. Aerosol OT reverse micelles are powerful

Table 1.5 Literature aggregation numbers for Aerosol OT.

ñ	Solvent	Temp.(°C)	Technique*	Reference
1	methanol	25	UC	Peri, 1969
1	methanol	30	NMR	Ueno et al., 1978
3	dioxane	NA	VPO	Eicke et al., 1975
5	chloroform	30	NMR	Ueno et al., 1978
6	t-amyl alcohol	25	UC	Peri, 1969
12	benzene	25	CAL	Tamura et al., 1981
13	bemzene	25	VPO	Ueno et al., 1977
15	benzene	25	VPO	Eicke et al., 1975
23	benzene	28	LS	Kitahara et al., 1962
13	benzene	30	NMR	Ueno et al., 1978
10	benzene	37	VPO	Hermann et al., 1979
13	benzene	37	VPO	Ueno et al., 1977
14	benzene	40	VPO	Kon-no et al., 1971
13	benzene	45	VPO	Ueno et al., 1977
21	toluene	25	LS	Peri, 1969
14	ethylene dibromide	25	LS	Peri, 1969
17	CCl_4	25	VIS	Peri, 1969
17	CCl_4	25	VPO	Ueno et al., 1977
17	CCl_4	25	LS	Kon-no et al., 1971
17	CCl_4	30	VPO	Ueno et al., 1977
17	CCl_4	37	VPO	Ueno et al., 1977
21	CCl_4	40	VPO	Kon-no et al., 1971
17	CCl_4	45	VPO	Ueno et al., 1977
21	pentane	40	VPO	Kon-no et al., 1971
17	cyclohexane	25	VPO	Ueno et al., 1977
39	cyclohexane	25	LS	Frank et al., 1961
17	cyclohexane	37	VPO	Ueno et al., 1977
18	cyclohexane	40	VPO	Kon-no et al., 1971
17	cyclohexane	45	VPO	Ueno et al., 1977
17	isooctane	25	VPO	Eicke and Rehak, 1976
24	isooctane	25	LS	Peri, 1969
25	isooctane	25	VIS	Peri, 1969
26	isooctane	25	UC	Peri, 1969
30	n-octane	25	LS	Frank et al., 1969
26	n-nonane	25	LS	Peri, 1969
27	n-nonane	25	VIS	Peri, 1969
37	n-decane	25	LS	Frank et al., 1969
28	n-dodecane	25	VIS	Peri, 1969
44	n-dodecane	25	LS	Frank et al., 1969
29	n-hexadecane	25	VIS	Ueno, 1969

* UC = ultracentrifugation, NMR = nuclear magnetic resonance, VPO = vapor pressure osmometry, LS = light scattering, VIS = viscosimetry, CAL = calculated;

Table 1.6 Literature critical micelle concentration values for Aerosol OT.

CMC $x10^{-3}$	Solvent	Temp.(°C)	Technique*	Reference
0.6	CCl_4	20	PS	Eicke, 1980
0.16	CCl_4	25	ITM	Ueno et al., 1977
0.16	CCl_4	25-35	VPO	Kitahara et al., 1978
0.24	CCl_4	30	ITM	Ueno et al., 1977
0.39	CCl_4	37	ITM	Ueno et al., 1977
0.6	CCl_4	NA	PS	Muto and Meguro, 1973
0.5	benzene	20	C	Tamura et al., 1981
0.9	benzene	20	PS	Tamura et al., 1981
2.0	benzene	20	PS	Eicke, 1980
2.2	benzene	22	PA	Jean and Ache, 1978
2.1	benzene	22	PA	Jean and Ache, 1978
0.4	benzene	25	VPO	Tamura et al., 1981
0.7	benzene	25	PS	Tamura et al., 1981
2.8	benzene	28	LS	Eicke, 1980
0.35	benzene	37	ITM	Ueno et al., 1977
2.3	benzene	37	VPO	Eicke, 1980
2.8	benzene	37	LS	Eicke, 1980
1.3	cyclohexane	28	LS	Eicke, 1980
0.33	cyclohexane	37	ITM	Ueno et al., 1977
0.39	cyclohexane	37	VPO	Eicke, 1980
1.1	cyclohexane	NA	PS	Fendler and Fendler, 1975
0.9	isooctane	22	PA	Jean and Ache, 1978
0.49	isooctane	25	LS	Eicke, 1980

* PS = probe spectroscopy, ITM = interfacial tension with mercury, VPO = vapor pressure osmometry, C = specific conductivity, PA = positron annihilation, LS = light scattering; NA = not available

agents for solubilization of water and other polar compounds into nonpolar solvents. As an example, 0.08 M Aerosol OT in isooctane can solubilize 4.8 M water (8.6 %) at room temperature (Zulauf and Eicke, 1979), a more than 10^3 increase over the solubility in pure isooctane. Reverse micelles can increase their size and aggregation number in order to encapsulate polar molecules which are larger in size than the micellar dimensions (Fletcher et al., 1984b).

Water has a substantial effect on the size and aggregation number of reverse micelles. The molar ratio of water to surfactant is designated in the literature as R (or w; or w_o). Table 1.7 shows the dramatic increase of micellar radius and aggregation number with increasing water content. Traces of moisture in the solvents and associated with the surfactant prohibit the preparation of completely water-free solutions (Eicke and Christen, 1978; Hermann and Schelly, 1979; Fendler, 1982). This water forms a hydrogen bond network with the surfactant polar head groups and helps to solvate the counterion in the micelle. As R is increased to six, the water molecules continue to form a solvation shell with the polar head groups and the counterions (bound water). Above an R of six, water is no longer required to solvate the surfactant and instead begins to form a "water pool" in

Table 1.7 Effect of added water on the size and aggregation number of Aerosol OT reverse micelles in cyclohexane.

R	$\tilde{n}$	radius(nm)
1	27	64
2	36	81
3	47	100
4	59	119
5	72	137
6	86	154
7	101	171
8	114	186

Data adapted from Day et al. (1979).

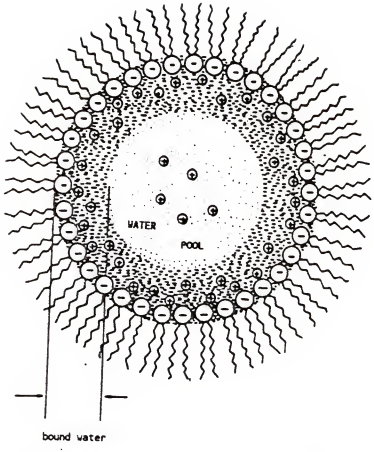


Figure 1.4 Schematic of an Aerosol OT reverse micelle showing the bound water region and the water pool. Adapted from El Seoud, 1984.

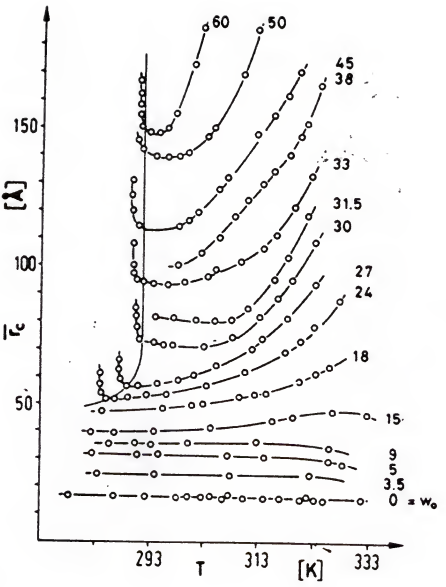


Figure 1.5 Effect of water and temperature on the size of Aerosol OT reverse micelles. The region below 18°C at higher water amounts is a phase separation. Adapted from Zulauf and Eicke, 1979.

the center of the micelle which creates an environment similar to that of bulk water. Figure 1.4 is a schematic of an Aerosol OT reverse micelle showing the bound and water pool regions. The micellar microenvironment results in a gradient of physical parameters, such as ionic strength, polarity, viscosity and acidity between the exterior and the interior of the aggregate.

Figure 1.5 shows the temperature dependence of micellar size at various R values for Aerosol OT reverse micelles. For R values which are less than 10 to 15, the size is independent of temperature over a wide range. However, above an R of 15, aggregate size becomes dependent on temperature. This is the transition region to a water in oil microemulsion system in which aggregate size is controlled by physical parameters such as temperature and pressure (Eicke, 1980). At temperatures below 18°C and above R values of 24, a region of phase separation is observed.

The dynamics of reverse micelles are comparable to those of normal micelles; however, reverse micelles undergo an additional process termed "water pool exchange". Micellar collisions occur on the microsecond (10^{-6} s) time scale and approximately one in a thousand of these (10^{-3} s) result in a mixing of the respective water pool contents (Fletcher et al.,

1984a). Monomers and solutes exit from normal and reverse micelles in the microsecond to millisecond range. For example, El Seoud and Fendler (1975) found a residence time of 3×10^{-3} seconds for pyrazole in Aerosol OT micelles in carbon tetrachloride. Micellar formation can occur in 10^{-8} to 10^{-6} seconds and micellar breakup occurs in 10^{-3} to 10^{0} seconds (Turro et al., 1980).

Micellar Liquid Chromatography

Micellar liquid chromatography (MLC) is an example of a relatively recently developed technique; pseudophase liquid chromatography. In this technique, solutes partition into micellar or macromolecular additives in solution in the mobile phase which behave as though they were a distinct phase. Partition of the solutes into the pseudophase can be used to control their chromatographic retention. MLC was first demonstrated by Armstrong and Henry (1980).

The use of micelles in separations has been reviewed recently (Armstrong, 1985; Dorsey, 1987; Khaledi, 1988) and the proceedings of a symposium on organized media in separations has been published (Hinze and Armstrong, 1987). Although reverse micelles in nonpolar solvents have been used for thin layer chromatography in several instances (Armstrong and

Terrill, 1979; Armstrong and McNeely, 1979), nearly all MLC has been done using normal micelles and only Hernandez-Torres et al. (1986) have used reverse micelles in HPLC. Recently an effort has been made to use reverse micelles in supercritical fluid chromatography because the solvent characteristics of these fluids are similar to those of nonpolar solvents such as alkanes (Gale et al., 1987a; Gale et al., 1987b).

In micellar RPLC minor advantages such as decreased toxicity and lower cost when compared to hydro-organic mobile phases are coupled with more substantial attributes such as unique selectivities and, in certain systems, enhanced detection sensitivity (Hinze et al., 1984; Skrilec et al., 1980; Cline Love et al., 1981).

Of particular interest for NPLC, where solvent demixing and excessive reequilibration times severely limit it's application, are the special advantages that MLC provides when used for gradient elution. Dorsey et al. (1984) and Landy and Dorsey (1984) have demonstrated that the reequilibration time in gradient elution RPLC is eliminated when micelles are used in place of hydro-organic modifiers. This is a result of the constancy of monomer concentration and surfactant

loading onto the stationary phase as the surfactant concentration is varied above the CMC.

In addition, Hernandez-Torres et al. (1986) have shown that MLC can be used to alleviate a traditional problem in NPLC, that of deactivation of the stationary phase by trace moisture in the mobile phase.

Figure 1.6 is a schematic of the processes occurring in MLC. Arunyanart and Cline Love (1984) have derived an equation relating chromatographic retention to micellar concentration. In liquid chromatography the capacity factor, k, is defined as the ratio of the amount of solute in the stationary phase to the amount of solute present in the mobile phase. For the case of MLC, the amount of solute in the mobile phase includes that solute which is in the micelles

$$k = \frac{\phi\ [S_{sp}]}{[S_{mp}] + [S_{mic}]} \qquad \text{(eqn. 1.8)}$$

where ϕ is the volume phase ratio, and $[S_{sp}]$, $[S_{mp}]$ and $[S_{mic}]$ are the solute concentrations in the stationary phase, mobile phase and micelles respectively. Incorporation of the binding constants for the solute between the micellar phase and the mobile phase, K_m, and between the mobile phase and the stationary phase, K_s,

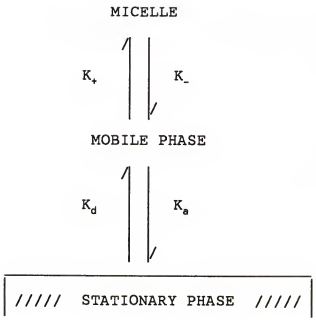


Figure 1.6 Schematic diagram of the partition processes for micellar liquid chromatography. K_+ and K_- are the entrance and exit constants into and out of the micelle. K_a and K_d are the adsorption and desorption constants onto and off of the stationary phase.

$$K_m = K_+/K_- \qquad \text{(eqn. 1.9)}$$

$$K_s = K_a/K_d \qquad \text{(eqn. 1.10)}$$

(see Figure 1.6) results in

$$\frac{1}{k} = \frac{[MIC]K_m}{\phi[SP]K_s} + \frac{1}{\phi[SP]K_s} \qquad \text{(eqn. 1.11)}$$

where [MIC] is the concentration of micellar surfactant and [SP] is the concentration of stationary phase sites. This equation predicts an inverse linear relationship between micellar concentration and the capacity factor, a result which has been confirmed experimentally for micellar RPLC (Arunyanart and Cline Love, 1984; Khaledi et al., 1987; Khaledi, 1988).

The micellar contribution to solute retention consists of two parts; the propensity of the solute to bind with the micelle, which controls selectivity, and the concentration of micelles present, which can be varied to control elution strength. If the slope and intercept of a 1/k versus [MIC] plot are ratioed, these two effects can be mathematically isolated and the binding constant per surfactant monomer can be obtained. This can be multiplied by the aggregation number to obtain the micellar binding constant.

A major drawback in the use of MLC has been the substantial loss in efficiency obtained with these systems. The decreased chromatographic efficiency is generally attributed to slow desorption of the solute from the stationary phase which is coated with a layer of adsorbed surfactant. The use of increased separation temperatures and addition of small amounts of short chain alcohols in the mobile phase to wet the stationary phase have been recommended to alleviate this problem (Dorsey et al., 1983). Hernandez-Torres et al. (1986) have observed a similar efficiency decrease for reverse micellar NPLC.

A further problem with MLC in reversed phase systems is the low elution strength attainable with the micellar modifier as compared to hydro-organic modifiers. Because of the highly polar nature of the interior of reverse micelles, it is expected that micellar NPLC may provide a stronger eluent than that found for micellar RPLC.

CHAPTER 2

ADSORPTION ISOTHERMS OF AEROSOL OT SURFACTANT ON CHROMATOGRAPHIC STATIONARY PHASES

Introduction

The equilibrium distribution of a mobile phase component between the mobile phase and the stationary phase is of fundamental importance for chromatographic systems. In particular, for NPLC systems where the mechanism of retention is adsorption, the effect of component concentration on this distribution (isotherm) is of considerable interest. If, for example, the mobile phase component of interest is a solute, nonlinearity of the isotherm can lead to diminished sample capacity, lowered chromatographic efficiency, chromatographic band asymmetry and retention times that shift with solute concentration.

The adsorption of other mobile phase components such as modifiers or inadvertently present water can also have a profound effect on the chromatographic system. Usually, modifiers are used to adjust eluent strength since they compete for adsorption sites with the solutes. However, often small amounts of polar modifiers such as alcohols or water are added to NPLC

systems that employ stationary phases which exhibit a high degree of surface heterogeneity (underivatized silica or alumina) to block high energy adsorption sites. These high energy sites cause nonlinear isotherms and slow desorption kinetics for solutes during the chromatographic process. The adsorption of these modifiers onto the stationary phase can therefore provide improved chromatographic efficiency, symmetry, capacity and retention time stability.

The adsorption and desorption of mobile phase modifiers is also of considerable importance in gradient elution chromatography. During the gradient, as the concentration of modifier is raised to increase eluent strength, selective adsorption of the modifier onto the stationary phase occurs (solvent demixing), decreasing it's concentration in the mobile phase and resulting in a weakened mobile phase. Following gradient elution, the chromatographic system must be returned to its initial equilibrium condition prior to initiating a new analytical run. However, slow desorption of the modifier from the stationary phase causes reequilibration time to be excessive. In practice, this long reequilibration time requirement for NPLC systems severely limits their application for gradient elution.

As a result of the implications for all aspects of chromatographic performance, adsorption of mobile phase components onto stationary phase materials has received considerable attention. In NPLC systems, the adsorption of water, modifiers and solutes has been studied by several workers (Eltekov et al., 1985; Thomas and Eon, 1977; Jacobson et al., 1984; Rizzi, 1985; Souteyrand et al., 1983; Paanakker et al., 1978; Scott, 1980). The adsorption of surfactants in ion-pair and micellar RPLC systems is also of interest (Berthod et al., 1986; Tramposch and Weber, 1986; Lin et al., 1983; Girard and Gonnet, 1985; Stahlberg and Hagglund, 1988; Borgerding and Hinze, 1985). Dorsey et al. (1984) have demonstrated that as a consequence of the constancy of surfactant monomer concentration above the CMC, adsorption isotherms of sodium dodecyl sulfate in micellar RPLC systems exhibit a region of constant surfactant loading for concentrations above the CMC. This property allows micellar gradients to be used with the need for reequilibration time eliminated.

In a typical NPLC system several factors are important in determining the loading and concentration dependence of adsorption of mobile phase components. These include:

a. The number of available adsorption sites.

b. The energy and energy distribution of these adsorption sites.

c. The adsorption affinity of the component.

d. The relative adsorption affinity of other mobile phase components.

e. Solution interactions between the component and other mobile phase components.

f. Interactions between the component and mobile phase components adsorbed on the stationary phase.

g. Self-association (aggregation) of the component in solution.

In addition, for the case of an ionic component, electrostatic interactions such as repulsion from a similarly charged surface may be important.

Giles (1961; Giles and Easton, 1966) has devised a classification scheme for adsorption isotherms. The four common types are designated S, L, H and C (Figure 2.1). An S-type isotherm results from cooperative adsorption; adsorbed solute molecules facilitate the adsorption of other solute molecules. An L-type isotherm (Langmuir) results from decreasing solute adsorption at higher concentrations as more of the adsorption surface is blocked by adsorbed solute molecules. H (high affinity) isotherms obtained in chromatographic systems are usually the result of limited availability of adsorption sites combined with strong adsorption of the solute (Snyder, 1968). C-type isotherms exhibit a constant adsorption distribution as the concentration is increased and are indicative of

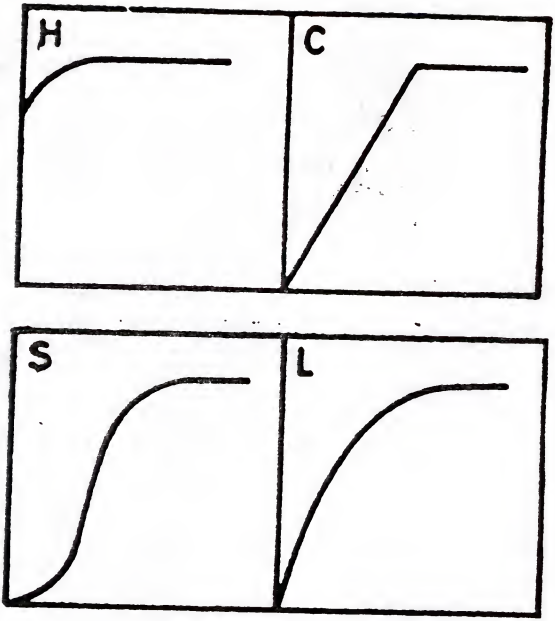


Figure 2.1 Giles' classification system of adsorption isotherms. X-axis is solute concentration in liquid phase and y-axis is solute concentration on the solid phase. See text for discussion. Adapted from Giles and Easton, 1966.

systems where adsorption causes fresh adsorption sites to become available (for example, adsorption causes the solid surface to swell exposing additional adsorption sites).

All of these isotherm types exhibit a saturation plateau at higher concentrations and can also exhibit a region of increased adsorption at still higher concentrations indicative of multilayer formation.

Frontal Chromatography

In order to study component adsorption in systems of chromatographic interest, often the chromatographic method itself provides the most convenient technique. Frontal chromatography is the chromatographic method most often used to determine adsorption isotherms (Berthod et al., 1986; Eltekov et al., 1985; Chuduk et al., 1981; Dorsey et al., 1984; Paanakker et al., 1978).

Wang et al. (1978) compared frontal chromatography with the static (shake flask) approach for determining the adsorption isotherm of 1-hexanol in hexane on silica. They obtained good agreement between the two techniques with the dynamic (chromatographic) technique being more convenient.

Jacobson et al. (1984) have undertaken a comparison study of several chromatographic techniques

for determination of adsorption isotherms in NPLC systems. They concluded that frontal chromatography was the most accurate and convenient chromatographic method for measuring isotherms.

In frontal chromatography, a solution containing a known concentration of the solute (C_l) is passed through a column containing a known amount of the adsorbent (W_a) at a constant flow rate (F). The column effluent is monitored by an appropriate detection system. As the solution passes through the column, solute is removed by the adsorbent until equilibrium occurs. A breakthrough front (Figure 2.2) is obtained indicating that solute equilibrium between the solution and the adsorbent has been achieved. The retention time of this front (T_f, the time at the half-way point of the breakthrough front) can be used to calculate the equilibrium concentration of the solute on the adsorbent (C_a);

$$V_f = T_f F \qquad \text{(eqn. 2.1)}$$

$$C_a = \frac{C_l(V_f - V_o)}{W_a} \qquad \text{(eqn. 2.2)}$$

where V_f and V_o are the front retention volume and system void volume respectively. This equilibrium

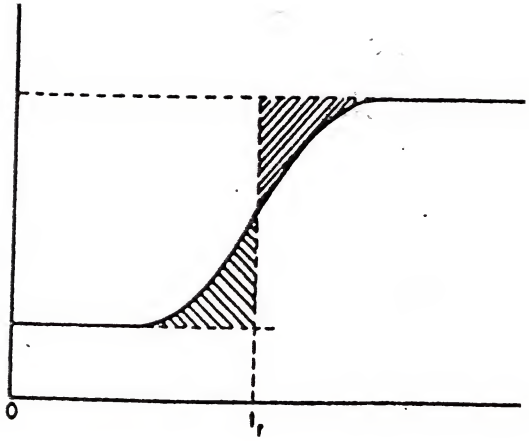


Figure 2.2 Typical frontal chromatogram showing breakthrough front and front retention time T_f. X-axis is time and y-axis is absorbance of the column effluent. Adapted from Wang et al., 1978.

concentration (in mol/g) can be converted to μmol/m^2 by dividing by the adsorbent surface area (in m^2/g) and performing the appropriate unit conversions.

Following equilibration at one concentration, the mobile phase composition can be step-increased and the equilibrium distribution at a second concentration determined. In this case, the equilibrium concentration of the additional adsorbed solute (C_{a2}) determined by equation 2.2 is added to the adsorbed concentration from the previous concentration (C_{a1}) to obtain the adsorbed equilibrium concentration at the second concentration (C_{2a}).

$$C_{2a} = C_{a1} + C_{a2} \qquad \text{(eqn. 2.3)}$$

Experimental Section

Apparatus Figure 2.3 is a block diagram of the HPLC system used in these experiments. The HPLC pump was an Altex (Berkeley, CA) 110A equipped with a 0.45 micrometer inlet filter, a pulse dampener and a drain valve. An Altex 210A injection valve with a 20 microliter loop was used. (The injection loop was used for experiments to determine the effect of surfactant concentration on solute retention which were done concurrently with the isotherm experiments.) Altex

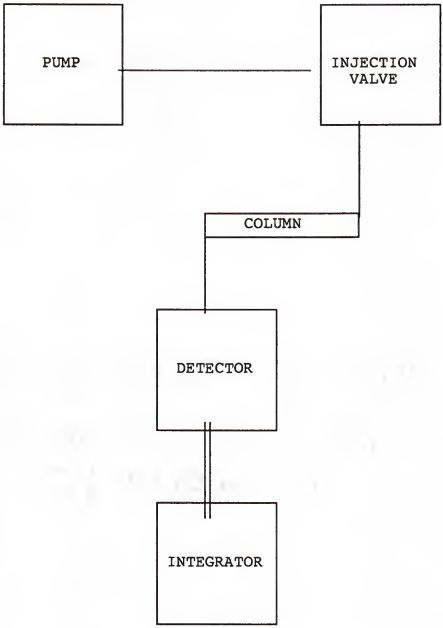


Figure 2.3 Block diagram of the HPLC system used for the frontal chromatography determination of adsorption isotherms.

Ultrasphere 5 micrometer, 250 x 4.6 millimeter cyano and silica columns were used and solute elution was monitored with a Perkin Elmer (Norwalk, CT) LC-75 variable wavelength ultraviolet detector with an 8 microliter flow cell. The column was maintained at 30°C by use of a glass column jacket and a Haake Buchler Instrument, Inc. (Saddle Brook, NJ) L/D1 constant temperature circulating bath. Data was acquired using a Shimadzu Instrument Co. (Kyoto, Japan) C-R3A integrator. Pump flow rate was calibrated by use of a stopwatch.

Reagents Hexane and isopropyl alcohol high purity solvents were obtained from Burdick and Jackson (Muskegon, MI) and dried using Davison (Baltimore, MD) 3A, 8-12 mesh molecular sieves. Water was obtained from Burdick and Jackson (high purity solvent). Aerosol OT was obtained from Fisher Scientific Co. (Pittsburgh, PA). It was sliced into thin (1 millimeter) sheets, dried in a circulating oven at 150°C for eight hours and stored in a desiccator.

Procedure Frontal chromatography was used to measure Aerosol OT adsorption isotherms at 30°C in the concentration ranges from 0.0001 to 0.2 M. Adsorption on a silica column was determined using four different mobile phases; hexane, 0.1% water in hexane, 3% isopropyl alcohol in hexane and water (R = 10) in

hexane. Adsorption on the cyano bonded phase column was measured using a hexane mobile phase.

Mobile phases were formulated by adding volume/volume amounts of isopropyl alcohol or water to the hexane to provide the desired concentration. Aerosol OT was weighed out and dissolved in the solvent prior to water addition. Solvents were stored over molecular sieves and passed through Millipore (Bedford, MA) 0.45 micrometer Mitex filters just prior to use. The solvent reservoir was equipped with a drying tube (a 30 centimeter length of 10 millimeter id. tygon tubing packed with Drierite (W. A. Hammond, Xenia, OH) on the air intake.

The detection wavelengths used ranged from 215 to 250 nanometers. Shorter wavelengths were needed to provide the required detector sensitivity at the lowest surfactant concentrations tested and longer wavelengths were required to prevent detector overload at the higher concentrations. Mobile phase flow rates ranged from 0.2 to 2.0 milliliters/minute (ml/min). Higher flow rates were used at the lowest concentrations, where breakthrough times as long as 20 hours were observed and slower flow rates were used at the higher concentrations to minimize experimental error.

The weight of stationary phase packing material in a 25 centimeter column was taken to be 4.17 grams and

the surface area of the Ultrasphere packing material is 300 m^2/g (Cooke, 1988). The column void volumes were taken as the point of first deviation from baseline of the solvent front.

Results and Discussion

Practical Considerations

Adsorption measurements at low solute concentrations using frontal chromatography are subject to a low degree of error. In examining equation 2.2, it can be seen that the retention volume (V_f) will be a large number (several thousand milliliters at 0.0001M) with a low degree of error and will be much larger than the void volume (V_o). The mobile phase concentration (C_l) is also a small number and this helps to minimize the effect of any errors in V_f.

In contrast, the potential for error increases with the solute concentration. The retention volume decreases rapidly and approaches the void volume. Since the error associated with measuring retention time is approximately the same regardless of the magnitude of the absolute value, the relative error in these measurements will be increased at shorter retention times. Also the void volume becomes similar in magnitude to the retention volume and can also contribute to the error. Finally and most importantly,

as the mobile phase concentration is increased, errors in retention volume translate into much larger errors in the calculated adsorbent concentration since the mobile phase concentration is multiplied by V_f.

In order to minimize these potential sources of error at high concentrations, lower mobile phase flow rates were employed. For mobile phase concentrations at or above 0.02 M and for determination of the void volume, flow rates of 0.2 ml/min were used.

Adsorption Isotherms

Figure 2.4 shows the adsorption isotherms of Aerosol OT obtained with four different mobile phases on the silica column. Figure 2.5 is a plot of the same isotherms using the logarithm of solution concentrations for clarity at the lower solute concentrations.

Hexane Mobile Phase on Silica The isotherm for the hexane mobile phase is of the Giles H-type. The surfactant loading level rises only slightly throughout the tested concentration range (0.0001 to 0.2M) from 0.57 to 0.69 $\mu mol/m^2$.

These results are consistent with the picture of the silica surface as having a limited number of available silanol adsorption sites and with a strong adsorption affinity of the Aerosol OT for these sites.

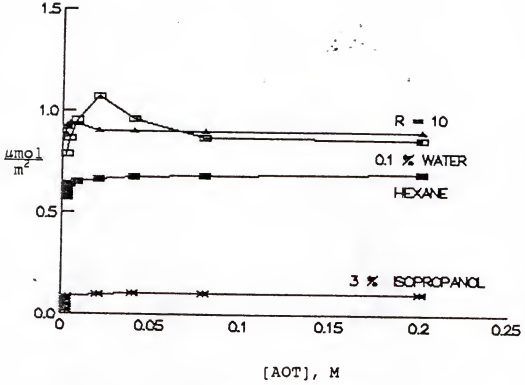


Figure 2.4 Experimentally observed adsorption isotherms of Aerosol OT on an Ultrasphere silica stationary phase at 30°C. The four mobile phases were: hexane, hexane with added water ([water]/[AOT] = 10), hexane with added water (0.1 %), and hexane with added isopropyl alcohol (3 %). The tested surfactant concentration range was 0.0001 to 0.2 M.

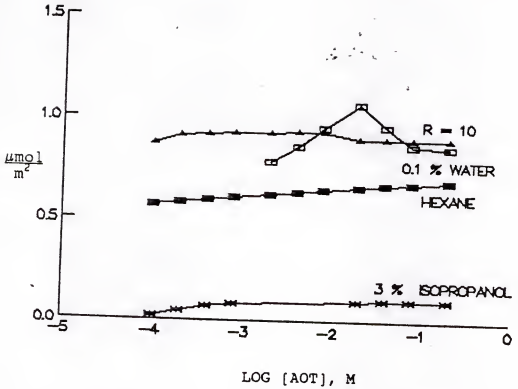


Figure 2.5 Semilog plot of the adsorption isotherms shown in Figure 2.4.

Near saturation of the available sites occurs even at the lowest concentration tested.

The strong interaction of silanol sites with solutes occurs because the silanols behave as a hydrogen bond donor. Therefore the basicity of functional groups in the solute is important in determining adsorption affinity for the silica. It has long been known that the presence of sulfonate groups in a solute increases it's strength of adsorption (Giles, 1961). El Tayar et al. (1988) have examined the adsorption strength on silica of 27 monosubstituted benzene compounds containing a wide variety of functional groups (alcohol, amine, halogen, ether, aldehyde, ketone, ester, nitro, cyano, amide and thio ether). They showed that a good correlation existed between adsorption strength and hydrogen bond acceptor basicity. The most strongly adsorbed compounds of those tested were those that contained sulfonyl groups. Ester compounds were also strongly adsorbed. Since Aerosol OT contains both sulfonyl and ester functional groups, it would expected to adsorb strongly onto silica silanol sites.

The availability of a limited number of strong silanol sites on the silica surface is widely accepted (Snyder, 1968; Snyder and Ward, 1966; Souteyrand et al., 1983). Snyder (1968) reports that as a general

rule, the maximum loading level of an organic compound onto a chromatographic adsorbent is about 0.0003 g/m^2 or 0.68 μmol/m^2 for the case of Aerosol OT with a molecular weight of 444 g/mol. This is in close agreement with the value obtained. Souteyrand et al. (1983) calculated that there were 3.6 μmol/m^2 of available silanols for water as a solute on a Lichrosorb SI 60 column which had a surface area of 482 m^2/g.

Table 2.1 lists some literature saturation loading values for NPLC systems and for micellar RPLC systems. The values range from 1 to 4.5 μmol/m^2 in the NPLC systems with a typical value of about 2 μmol/m^2. The micellar RPLC systems have values between 0.5 and 2 μmol/m^2, which may reflect the larger size of these solutes and/or the coverage of the adsorbent with the hydrocarbon bonded phase.

Aerosol OT is a relatively large molecule and would be expected to attain a lower loading value than the solutes in the NPLC systems in Table 2.1. A solute such as 1-hexanol occupies only a small region of the adsorbent surface since it orients in an end-on position. Aerosol OT has a large polar head containing the two ester groups separated by methyl groups from the sulfonate group in the center and it is probable that the molecule undergoes multi-site adsorption. It

Table 2.1 Literature values for adsorption saturation on chromatographic stationary phase materials for NPLC and micellar RPLC.

solute	solvent	adsorbent	surface area (m^2/g)	loading value ($\mu mol/m^2$)	reference
anisole	heptane	silica	117	1	Chudek et al., 1981
butanol	octane	silica	400	2.4	Paanakker at al., 1978
benzene	heptane	silica	520	2.3	Eltekov et al., 1985
hexanol	hexane	silica	340	4.5	Wang et al., 1978
water	methylene chloride	silica	480	3.6	Souteyrand et al., 1983
SDS	water	ODS	235	1.8	Dorsey, et al., 1984
SDS	water	silica	150	2.0	Berthod, et al., 1986
CTAB	water	silica	150	0.5	Berthod, et al., 1986

SDS is sodium dodecyl sulfate, CTAB is cetyltrimethyl ammonium bromide and ODS is octyldecylsilane bonded phase on silica.

is a top-shaped molecule, as was discussed in Chapter 1, and the combination of the large polar head with the bulky branched alkyl chains would require a relatively large area on the adsorbent surface. In addition, silanol sites inside of smaller pores would not be accessible to such a bulky molecule. The lower loading value observed in this study therefore seems reasonable for this solute.

Water (R = 10, 0.1%) in Hexane Mobile Phase on Silica The adsorption isotherm obtained with added water at an R value of 10 is very similar to the one with the hexane mobile phase. It is of the H-type with the loading value nearly constant throughout the tested concentration range. The loading value is shifted higher than that found with pure hexane to about 0.93 $\mu mol/m^2$.

We believe that the additional loading occurs because the surfactant is adsorbed onto a layer of water molecules on the adsorbent surface. Adsorption sites which were not accessible to the surfactant are accessible to the water molecule and the backside of the adsorbed water is then accessible to the surfactant because it is further out from the surface.

Paanakker et al., (1978) have studied the adsorption of 1-butanol in isooctane onto chromatographic silica in the presence and absence of

added water. They found that the loading of butanol onto the silica was unchanged by the addition of even large amounts of water. Rizzi (1985) has subjected these experimental results to a theoretical treatment, including the calculation of activity coefficients for all species. He concludes that the butanol is adsorbed onto the top of mono or multilayer water on the silica surface. In their system it appears that the size difference between water and 1-butanol is not sufficient to observe a noticeable increase in loading level.

The presence of a layer of adsorbed water on the silica indicates that an equilibrium is set up between micellar water and adsorbed water. This is not surprising since, as will be shown in Chapter 3, solutes in micellar NPLC exhibit an analogous equilibrium behavior.

Further evidence of such an equilibrium is available from the results of an experiment in which a constant amount of water (0.1%) was added while the surfactant concentration was varied. This experiment was only conducted at surfactant concentrations at 0.002 M and above due to the limited solubility of water. In this experiment, the R value varied with surfactant concentration.

What was observed was an increased loading of surfactant as the concentration was increased up to 0.02 M. Loading levels as high as 1.07 $\mu mol/m^2$ were observed. Above 0.02 M, the loading values observed were identical to those found in the R = 10 experiment. It is believed that at the lower surfactant concentrations increased surfactant loading is due to higher R values and additional adsorbed water on the silica. This evidence supports the concept of a dynamic equilibrium between the various water species in these systems.

Isopropyl Alcohol (3%) in Hexane Mobile Phase on Silica The adsorption isotherm in the presence of 3% added isopropyl alcohol indicates a competition for adsorption sites is occurring between the alcohol and surfactant. The loading value rises from approximately 0.02 to 0.1 $\mu mol/m^2$ over the tested concentration range. The relative adsorption affinity of Aerosol OT does not appear to be comparable to that of the isopropyl alcohol, since the molar ratio of alcohol to surfactant decreases from 40,000 to 2 over the tested range and yet the surfactant loading does not increase substantially. A backside adsorption of the surfactant onto the adsorbed alcohol layer analogous to that seen with the water system would not be expected to occur.

Hexane Mobile Phase on Cyano Bonded Phase Figures 2.6 and 2.7 are the experimental adsorption isotherms found for Aerosol OT in hexane on a cyano bonded phase column at 30°C. The isotherm is also of the Giles H-type. Surfactant loading rises from 0.16 to approximately 0.6 $\mu mol/m^2$ over the tested concentration range.

Snyder (1983) has shown, that on cyano bonded phases, the primary adsorption sites are the silanols and not the cyano groups and that only about 20% of the silanols on the original underivatized silica are available to solutes. This means that only about 0.14 $\mu mol/m^2$ of these sites are available in this case given the results obtained on the bare silica (0.69 $\mu mol/m^2$). This is in good agreement with the loading found at the lowest concentration tested, indicating that the available silanols are saturated at this concentration.

Additional adsorption at higher concentrations appears to occur onto the cyano sites. These sites are relatively weak and this is manifested by the corresponding small increase in surfactant loading throughout the tested concentration range.

Implications for Micellar NPLC

A number of conclusions can be drawn from the results of these investigations of surfactant adsorption. The surfactant adsorbs strongly onto the

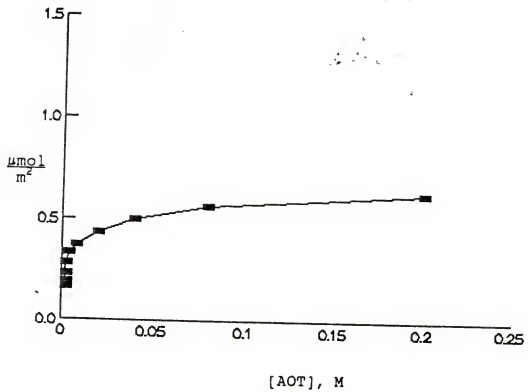


Figure 2.6 Experimentally observed adsorption isotherm of Aerosol OT on an Ultrasphere cyano bonded stationary phase using hexane as the mobile phase at 30°C. The tested surfactant concentration was 0.0002 to 0.2 M.

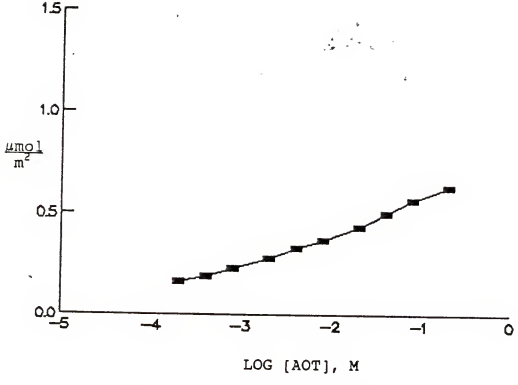


Figure 2.7 Semilog plot of the adsorption isotherm shown in Figure 2.6.

silanol sites available on the chromatographic surface. It does not adsorb as strongly as water, but instead adsorbs onto a layer of adsorbed water when water is added to the mobile phase. A dynamic equilibrium between micellar water and water adsorbed on the stationary phase appears to occur. The surfactant also does not adsorb as strongly as isopropyl alcohol, however the nature of isopropyl alcohol precludes a backside adsorption on the alcohol layer.

The flatness of these adsorption isotherms on silica, both with and without water (as long as R is constant), indicates that these systems should be readily amenable to gradient elution. It also indicates that the observed decreases in solute retention as a result of increasing surfactant concentration are due to aggregation behavior of the surfactant in solution and not to changes occurring on the stationary phase. This will be examined in Chapter 3.

Some controversy has occurred with regard to the mechanism of decreased chromatographic efficiency in micellar RPLC. An analogous decrease has been observed for micellar NPLC (Hernandez-Torres et al., 1986). The constancy of the isotherm allows the efficiency to be evaluated at maximum surfactant loading on the stationary phase and yet at concentrations well below

the CMC. This should provide a means to separately evaluate the effects due to slow mass transport at the adsorbent surface and those due to slow entrance/exit from the micelle. This problem will be examined in Chapter 4.

The adsorption isotherm on the cyano bonded phase column is different from that found on bare silica. In this case, the available silanol sites are saturated and then a slowly rising isotherm is observed corresponding to adsorption on weak cyano sites. It might be expected that gradient elution would be less feasible with this system. However, the isotherm slope is relatively shallow and the kinetics of desorption from these weak sites is expected to be rapid. So gradient elution with these systems might still be practical.

Other bonded stationary phases commonly used in NPLC, such as amino and diol, provide stronger adsorption sites than the cyano. Surfactant adsorption on these adsorbents would be expected to be intermediate between that observed for the two adsorbents examined here. Gradient elution would be expected to be more problematic for these bonded phases.

CHAPTER 3

SOLUTE RETENTION IN REVERSE MICELLAR NPLC

Introduction

As noted in Chapter 1, NPLC normally employs a nonpolar solvent (such as hexane) as the mobile phase solvent along with various amounts of a polar modifier (such as isopropyl alcohol) to adjust eluent strength. Eluent strength has a logarithmic dependence on the amount of polar modifier (see equation 1.4). Ideally, polar modifier molecules compete with the solute molecules for adsorption sites on the stationary phase and as their number is increased, solute retention decreases (equation 1.3).

This theory neglects solute-solvent and solvent-solvent interactions in the mobile phase and on the stationary phase and especially solute and solvent interactions with specific sites on the adsorbent. All of these interactions can provide contributions to solute retention. In general, as the polarity and hydrogen bonding ability of solutes and solvents increases, the contributions from these non-ideal interactions increases.

Retention in micellar liquid chromatography depends on the partition of the solute into the micelles, and eluent strength is controlled by the surfactant concentration (number of micelles). In general, more polar solutes and more nonpolar bulk solvents will enhance solute partition into reverse micelles (Fendler, 1976).

Equation 1.11 (p. 37), which predicts an inverse linear relationship between solute capacity factor and micellar concentration, has been experimentally verified for micellar RPLC (Arunyanart and Cline Love, 1984; Khaledi et al., 1987; Khaledi, 1988). Plots of this type provide a test for conformity of the system with pseudophase chromatographic theory and can also provide information about the partitioning of the solute into the micelle.

Armstrong (1985) has compiled a listing of theoretical expressions applicable to pseudophase liquid chromatography and has shown that they are all equivalent. Of particular interest is an expression for HPLC which was proposed and experimentally verified by Armstrong and Nome (1981):

$$\frac{V_r}{V_r - V_o} = \frac{\tilde{v}(K_{pm} - 1)\,[Mic]}{K_{ps}} + \frac{1}{K_{ps}} \qquad \text{(eqn. 3.1)}$$

where v_r is the solute retention volume, v_o is the system void volume, $\bar{v}$ is the partial specific volume of the surfactant in the micelle, [Mic] is the concentration of micellar surfactant (surfactant concentration - CMC), K_{pm} is the solute partition coefficient between micelle and the mobile phase, and K_{ps} is the solute partition coefficient between the stationary phase and the mobile phase.

Equations 1.11 and 3.1 are related by

$$K_m = \bar{v}(K_{pm} - 1) \qquad \text{(eqn. 3.2)}$$

and

$$K_{ps} = [SP]K_s \qquad \text{(eqn. 3.3)}$$

Partition coefficients (K_{pm}) are dimensionless while binding constants (K_m) have units of M^{-1} (Armstrong and Stine, 1983). In a manner analogous to that for equation 1.11, the left-hand side of equation 3.1 can be plotted versus micellar surfactant concentration and the linear result can be used to evaluate the partition coefficient. As with binding constants, the partition coefficient must be multiplied by the aggregation number to obtain the micellar partition coefficient.

Equations 1.11 and 3.1 provide equivalent information since they are interconvertible via

equation 3.2. In practice, the Armstrong equation requires information about the surfactant molar volume which is often not readily available and consequently equation 1.11 is more convenient to use.

Hernandez-Torres et al. (1986) have studied the effect of surfactant concentration on solute retention in two NPLC systems; hexane mobile phase on a silica column and on an amino bonded phase column. They observed two regions of dependence of solute retention on surfactant concentration. Below the CMC, there is a region where solute capacity factor depends very little on surfactant concentration. Above the CMC, solute retention decreases rapidly as the surfactant concentration is increased. Extrapolation of the two linear components of a log-log plot of capacity factor versus surfactant concentration allows the CMC to be obtained from the intersection.

Dorsey et al. (1983) have used this method to obtain CMC values for surfactants used in micellar RPLC and shown favorable comparison with literature values. Hernandez-Torres et al. (1986) obtained a value of 0.0035 M for Aerosol OT in dry hexane at 30°C which also shows good comparison with literature values. As noted in Chapter 1, a number of factors can influence CMC values for reverse micelles and the obtained values

should be considered "operational CMC's" for the system.

Evaluation of the effect of surfactant concentration on solute retention can provide physicochemical information about the micelles such as binding constants and CMC's. It can also be useful in determining the effect of variables such as solvents, solutes, polar modifiers and temperature on the micellization process. This aspect is especially important for reverse micellar NPLC where micelle formation can be influenced by all of these factors. The most dramatic example of this is provided by Fryar and Kaufmann (1969) who have shown that as little as 1% of methanol in toluene completely inhibits reverse micelle formation by barium dinonylnaphthalene sulfonate.

The flat adsorption isotherms observed for Aerosol OT in NPLC systems (Chapter 2) are conducive to gradient elution chromatography. It is of interest, therefore, to evaluate the elution strength of reverse micelles for NPLC. Snyder (1968) has derived an expression which can be used to compare the elution strengths of different mobile phases in NPLC:

$$\ln(k_1/k_2) = A_x(e_2^o - e_1^o) \qquad \text{(eqn. 3.4)}$$

where k_1 and k_2 are the capacity factors and e_1^o and e_2^o are the eluent strengths of mobile phases 1 and 2 respectively, and A_x is the molecular area of the solute. Dorsey et al. (1984) have used equation 3.4 to evaluate the elution strength of micellar mobile phases in RPLC.

The adsorption competition mechanism that is the theoretical foundation for equation 3.4 is totally nonapplicable to micellar NPLC (as it is to micellar RPLC). However, the estimation of eluent strengths of reverse micellar mobile phases should still allow a valid comparison with the existing and well established elution strength system (see Table 1.2 and Figure 1.1).

Experimental Section

Apparatus The HPLC system used in these experiments is described in Chapter 2.

Reagents The reagents used in these experiments are also described in Chapter 2.

Solutes TNT (2,4,6 trinitrotoluene) and RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) were obtained from the U. S. Army (Aberdeen, MD). Methomyl (5-methyl N-[(methylcarbomyl)oxy]thioacetimidate) was obtained from Chem Service (West Chester, PA). PNA (p-nitroaniline) was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI). BNOH (ß-naphthol) was

obtained from Sigma Chemical Company (St. Louis, MO). All were used with no further purification.

Procedure These retention experiments were conducted concurrently with the frontal chromatography experiments used to measure adsorption isotherms. Following the appearance of the breakthrough front, which indicated that equilibrium had been achieved between surfactant in the mobile phase and on the stationary phase at each tested concentration, the retention of test solutes was determined.

The following systems were examined; a hexane mobile phase on a silica column, a hexane plus water (R = 10) mobile phase on a silica column, a hexane plus water (0.1%) mobile phase on a silica column, a hexane plus isopropyl alcohol (3%) mobile phase on a silica column, and a hexane mobile phase on a cyano bonded phase column.

All experiments were conducted at 30°C and with a mobile phase flow rate of 1.0 ml/min. Solute concentrations used were; TNT - 10 μg/ml, BNOH - 9 μg/ml, PNA - 39 μg/ml, RDX - 52 μg/ml and methomyl 45 μg/ml. Small amounts of methylene chloride or isopropyl alcohol (for the 3% isopropyl system) had to be added to the injection solvent to solubilize PNA and methomyl. Twenty μl of the solute solutions were injected into the HPLC system.

Mobile phases were formulated by adding volume/volume amounts of isopropyl alcohol or water to the hexane to provide the desired concentration. Aerosol OT was weighed out and dissolved in the solvent prior to water addition. Solvents were stored over molecular sieves and passed through Millipore (Bedford, MA) 0.45 μm Mitex filters just prior to use. The solvent reservoir was equipped with a drying tube (a 30 cm length of 10 mm id tygon tubing packed with Drierite (W. A. Hammond, Xenia, OH)) on the air intake.

Solute elution was monitored at wavelengths ranging from 230 to 260 nanometers. Void volume was taken as the point of first deviation from baseline of the injection front. Solute capacity factor, k, was calculated by

$$k = \frac{V_r - V_o}{V_o} \qquad \text{(eqn. 3.5)}$$

where V_r is the solute retention volume and V_o is the system void volume.

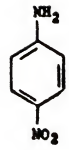
Results and Discussion

Solutes One of the objectives of this study was to evaluate the effect of solute type in reverse micellar NPLC. Aerosol OT is an anionic surfactant and there is evidence in the literature that the unique microenvironment which exists in these reverse micelles can substantially influence solute properties (Fendler,

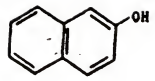
1982). For example, the ionization constants of acids are shifted in reverse micelles (Nome et al., 1976). Menger and Saito (1978) have observed an increase in the pKa of p-nitrophenol by 4.5 units in Aerosol OT reverse micelles in heptane. These effects are lessened as water is added to the reverse micelles.

In this work, BNOH was used as a representative acidic solute. It is a weak acid with a pKa of 9.5 at 25°C (CRC Handbook of Chemistry and Physics, 1984a). PNA was used as a representative basic solute. The nitro group in the para position is both resonance and inductively electron-withdrawing resulting in a weak base. The pKa of the anilinium ion is 4.63, while the value for the p-nitroanilinium ion is 1.0 at 25°C (CRC Handbook of Chemistry and Physics, 1984b). TNT was used as a neutral solute. In the 3% isopropyl alcohol system, RDX was used as a neutral solute and methomyl was used as a hydrogen bonding solute. The structures of these solutes are shown in Figure 3.1.

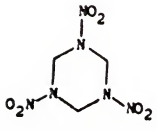
Solute Concentration Snyder (1968) has noted that NPLC systems often exhibit limited loading capacity. He has arbitrarily defined the loading capacity as that concentration at which solute capacity factor or efficiency exhibits a 10% deviation from that found at



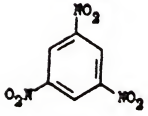
PNA



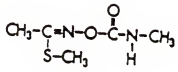
BNOH



RDX



TNT



Methomyl

Figure 3.1 Structures of solutes used in reverse micellar NPLC retention experiments. Adapted from The Merck Index (1976) and Analytical Reference Standards and Supplemental Data (1984).

lower concentrations. The retention of TNT and BNOH was monitored as concentration was varied to ascertain if the loading capacity was being exceeded. The system used for these experiments was a 0.0001 M Aerosol OT in 20% methylene chloride/80% hexane mobile phase on a silica column. As shown in Figure 3.2, TNT exhibited stable retention in the concentration range of 0.153 to 125 μg/ml. The retention of TNT showed a deviation greater than 10% at concentrations greater than 125 μg/ml. However, the concentration of TNT used in these experiments was ten times lower than this loading capacity limit. BNOH was tested from 1.03 to 206 μg/ml and showed stable retention over this range.

Effect of Surfactant Concentration on Solute Retention

Hexane Mobile Phase on Silica Figure 3.3 is a log-log plot of surfactant concentration versus solute capacity factor of a hexane mobile phase on a silica column. The tested surfactant concentrations ranged from 0.0001 to 0.2 M. PNA and BNOH exhibited two regions of retention dependence on surfactant concentration as expected. These plots are very similar to those found by Hernandez-Torres et al. (1986).

At the lowest concentrations, in the sub-micellar region, retention decreases only slightly with increasing surfactant concentration. The small

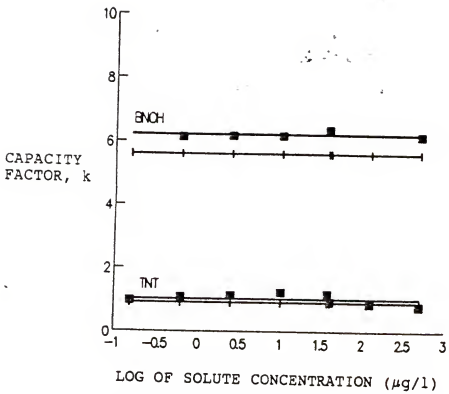


Figure 3.2 Effect of solute concentration on retention for a 0.0001 M Aerosol OT in 20 % methylene chloride/80 % hexane mobile phase on an Ultrasphere silica column at 30°C and 1 ml/min. The crosshatched lines represent a 10 % deviation in retention, indicating the limit of column capacity.

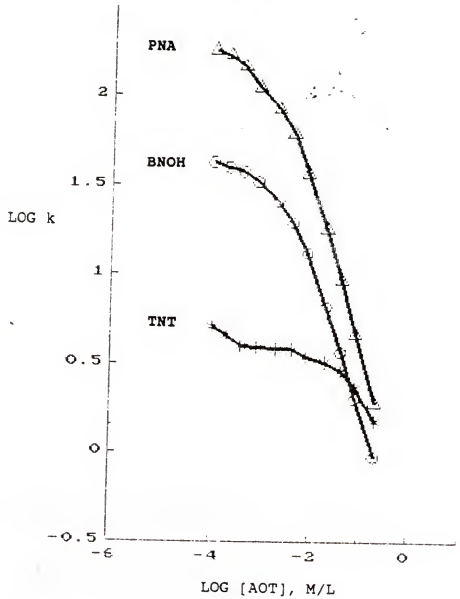


Figure 3.3 Effect of surfactant concentration on solute retention for a hexane mobile phase on an Ultrasphere silica column at 30°C.

decrease in retention in this region is probably not due to surfactant loading onto the stationary phase since (as was shown in Chapter 2) little additional loading is observed. Instead, the decreased retention is probably due to association of the solute with the surfactant in the mobile phase. This association may occur with the linear premicellar aggregates (LMPA's) that were discussed in Chapter 1.

In the region above the CMC, solute retention decreased rapidly and linearly with increasing surfactant concentration. This is consistent with partitioning of these solutes into the reverse micelles in the mobile phase.

TNT is a weakly retained solute in this system and did not exhibit the expected behavior. Instead, retention decreased fairly rapidly in the submicellar region, then leveled off and finally showed a substantial decrease only at the highest surfactant concentrations. The reasons for this behavior are unclear. As would be expected from polarity considerations, it appears that this solute does not readily partition into the reverse micelles. Consequently, retention for TNT is decreased only at the highest surfactant concentrations. The slope of the line for TNT is not as steep as for the other two solutes in this region and the final capacity factor

for TNT is higher than for BNOH. These observations also indicate lowered affinity of TNT for the reverse micelles.

Water (R = 10) in Hexane Mobile Phase on Silica

As noted in Chapter 1, when water is added to reverse micelles, the aggregation number and the micellar size increase dramatically. Day et al. (1979) have shown that the aggregation and size of Aerosol OT reverse micelles have little dependence on surfactant concentration or on the solvent (as long as it sufficiently nonpolar). These considerations allow the aggregation number and micellar size in the R = 10 in hexane mobile phase to be estimated by extrapolation of the data for cyclohexane given in Table 1.7. The resultant values are $\bar{n}$ = 137 and a radius of 224 nm.

The amount of water present in the reverse micelles will also affect solute partition. Increased water increases the polarity of the micellar interior and loosens the interfacial region (Fendler, 1976). It can be inferred from these considerations that water would therefore increase partitioning of polar solutes into the reverse micelles. However, Fendler (1976) indicates that more work needs to be done in this area.

The amount of water added in these experiments, provides a large water pool in the micellar interior. This amount of water represents the upper limit of the

reverse micellar region and at higher water amounts the system becomes a microemulsion.

The log-log plot for the R = 10 system is shown in Figure 3.4. The PNA and BNOH exhibit behavior very similar to that observed in the dry hexane system and these results indicate that this system behaves very predictably even with this large amount of water present.

The TNT also exhibits anomalous behavior in this system including a large decrease in retention at approximately 0.001 M surfactant. TNT also has regions of decreasing retention at both the lowest and highest concentrations similar to those seen in the dry hexane. These results appear to be caused by adsorption of water and surfactant onto the stationary phase at the lower concentrations and partitioning to the reverse micelles at the highest concentrations. The breaks in the curve may also be caused by association of TNT with certain stable LPMAs (see Chapter 1).

TNT has an increased affinity for these reverse micelles with the added water as evidenced by a steeper slope at the higher concentrations (although not as steep as for PNA and BNOH) and a lower k than BNOH and PNA at the highest concentration.

This experiment indicates that even with the addition of large amounts of water, the integrity of

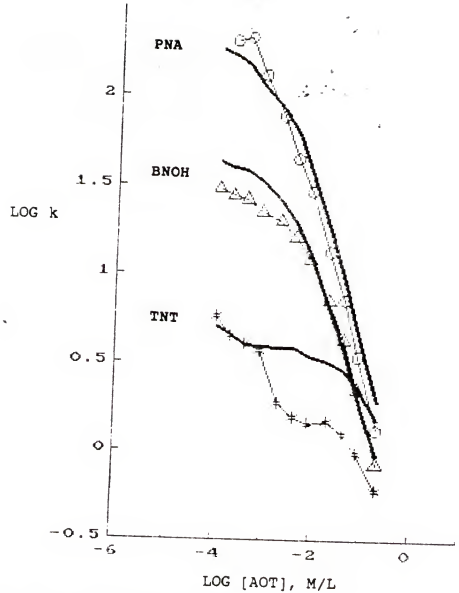


Figure 3.4 Effect of surfactant concentration on solute retention in a hexane with water added (R = 10) mobile phase on a Ultrasphere silica column at 30°C. The bold lines are for the dry hexane system for comparison purposes.

the reverse micelles is uncompromised and as a consequence of the encapsulation of this water by the reverse micelles, the NPLC system remains substantially unaffected.

Water (0.1%) in Hexane Mobile Phase on Silica As noted in Chapter 2, this experiment was conducted using a limited surfactant concentration range (0.002 to 0.2 M) because of the limited solubility of water in hexane. A consequence of maintaining a constant amount of water as the surfactant concentration was varied was that the R value was different at each surfactant concentration. The R value varied from 28 at 0.002 M to 0.3 at 0.2 M Aerosol OT.

Figure 3.5 shows the log-log plot observed in the 0.1% added water experiments. At 0.002 M surfactant, the retention of all three solutes was decreased by the water. This concentration is submicellar and with an R of 28, it appears that the water deactivated the stationary phase. At higher surfactant concentrations, the retention of PNA and BNOH is similar to that found in the hexane system indicating that the water is no longer deactivating the stationary phase. The adsorption isotherm for the 0.1% added water system (Figure 2.5) showed additional surfactant loading onto the stationary phase up to 0.02 M. Apparently the

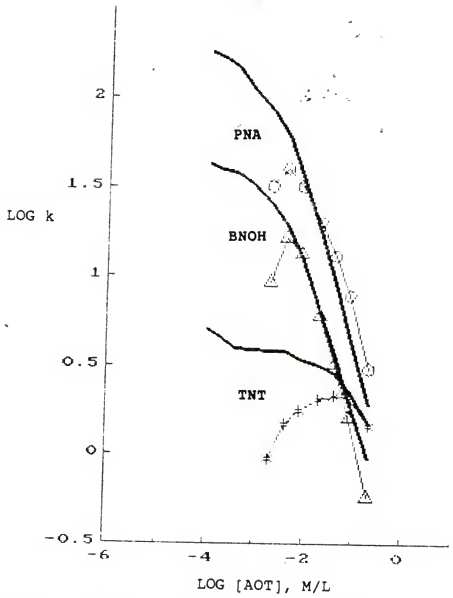


Figure 3.5 Effect of surfactant concentration on solute retention for a hexane with added water (0.1 %) mobile phase on an Ultrasphere silica column at 30°C. The bold lines are for the dry hexane system for comparison purposes.

additional adsorbed surfactant does not substantially influence the retention of PNA and BNOH.

TNT retention relative to the hexane system is decreased at all surfactant concentrations except the two highest tested. In conjunction with the results from the R = 10 system, this experiment indicates that the amount of water and surfactant adsorbed onto the stationary phase and not the micellar concentration primarily controls retention of this solute. While this is further evidence of weak partitioning of TNT to the reverse micelles, TNT is weakly retained in this system and this behavior probably cannot be generalized to other nonionic solutes.

Isopropyl Alcohol (3%) in Hexane Mobile Phase on Silica Polar modifiers, especially hydrogen bonding solvents, have an inhibitory effect on reverse micelle formation. It was of interest, therefore, to investigate solute retention in a system which contained 3% isopropyl alcohol. The results are shown in Figure 3.6.

Isopropyl alcohol and the reverse micelles appear to work in additive fashion to reduce solute retention. The PNA curve is similar to that found with hexane but the retention time at the submicellar concentrations has been substantially decreased by the modifier. This decrease in solute retention necessitated the use of

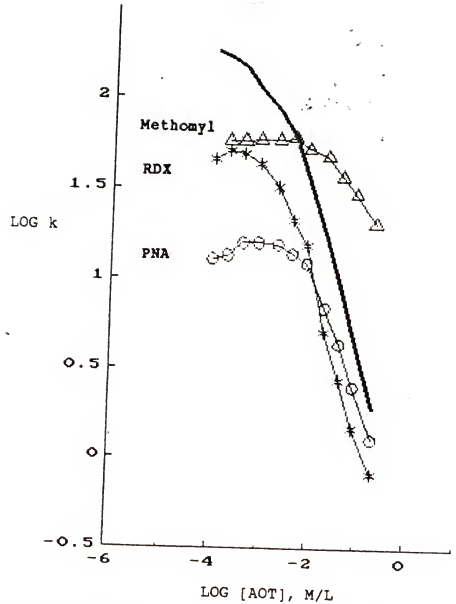


Figure 3.6 Effect of surfactant concentration on solute retention for a hexane with added isopropyl alcohol (3 %) mobile phase on an Ultrasphere silica column at 30°C. The bold line is that found for PNA in the dry hexane system.

more polar solutes than TNT and BNOH, which were only slightly retained.

This experiment provides indisputable evidence for the formation of reverse micelles in the presence of 3% isopropyl alcohol. Each of the solutes exhibits two regions of retention dependence with a rapid linear decrease as concentration is increased above the CMC. At the lowest concentrations, RDX and PNA exhibit increasing retention as surfactant concentration is increased. This may be due to increased adsorption of surfactant onto the stationary phase in this region (see Figure 2.5).

While the RDX and PNA curves are similar to what would be expected, the most polar solute, methomyl, appears to only weakly partition into the reverse micelles. The slope of the line above the CMC is not as steep as for the other solutes. The reason for this is probably due to an effect observed by El Seoud and Fendler (1975), where the binding constants of very polar solutes to reverse micelles were decreased because of solute self-aggregation in the nonpolar solvent. While the concentration of methomyl is quite low in these experiments, association of methomyl with the isopropyl alcohol is very probable and this would decrease partitioning to the reverse micelles.

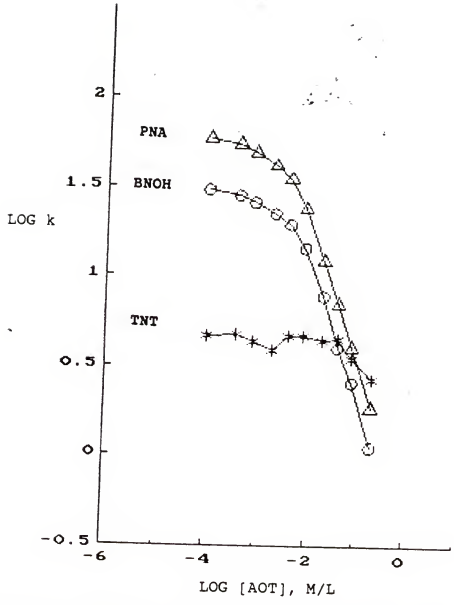


Figure 3.7 Effect of surfactant concentration on solute retention for a hexane mobile phase on an Ultrasphere cyano bonded phase column at 30°C.

Hexane Mobile Phase on a Cyano Bonded Phase Column

Figure 3.7 shows the effect of surfactant concentration on solute retention for a dry hexane mobile phase on an Ultrasphere cyano bonded phase column. The observed curves are very similar to those obtained on the silica column. The main difference is that the retention for PNA and BNOH is reduced at submicellar concentrations on the cyano column. This result is expected since the cyano stationary phase is weaker than the silica.

The other difference with regard to the silica system is that TNT retention is higher on the cyano column at higher surfactant concentrations. This provides further evidence that TNT retention is influenced more by stationary phase effects than by micellar concentration.

Elution Strength of Reverse Micellar Mobile Phases

Micellar mobile phases for use in RPLC are limited because they are relatively weak in terms of elution strength (Dorsey et al., 1984). From the results of these experiments, it appears that reverse micellar mobile phases may have the additional advantage of being fairly strong modifiers for NPLC. As an example, the retention of PNA in the dry hexane on silica system is decreased from about 450 minutes to 4 minutes as surfactant concentration is increased from 0.0001 to 0.2 M.

Equation 3.4 can be used to estimate the strength of the reverse micellar mobile phases. PNA was chosen as the comparison solute and it's molecular area was calculated as being 22.2 (8.5 $Å^2$ per unit) by the procedure and data provided by Snyder (1968). A 3% isopropyl alcohol mobile phase which provided a k of 13.6 was used as the reference mobile phase. The elution strength, e°, for the 3% isopropyl alcohol in hexane mobile phase was calculated using equation 1.4 and the value obtained was 0.47. The results obtained from use of equation 3.4 indicated that the e° of reverse micellar mobile phases were approximately 0.4 at the CMC and 0.6 at 0.2 M surfactant. Examination of Figure 1.1 indicates that the reverse micelles are comparable in strength to ethyl ether at the CMC and slightly stronger than acetonitrile at 0.2 M.

Elution strength is logarithmically related to the concentration of modifier for solvent mixtures. Micellar mobile phases have the additional advantage of having elution strength linearly related to surfactant concentration.

CMCs and Binding Constants Derived from Micellar Chromatography; Conformance with Pseudophase Liquid Chromatography Theory

As noted in the introduction, physicochemical data can be derived from these solute retention experiments.

Table 3.1 Critical micelle concentrations (M/L) for Aerosol OT at 30°C obtained by solute retention measurements in reverse micellar NPLC.

	SOLUTE			
SYSTEM	BNOH	PNA	RDX	METHOMYL
Hexane/Silica	0.0030	0.0036	------	------
Hexane/Silica/R=10	0.016	0.011	------	------
Hexane/Silica/3%IPA	------	0.0070	0.0024	0.010
Hexane/Cyano	0.0043	0.0039	------	------

Table 3.1 lists the CMC values obtained for Aerosol OT in these systems. The values obtained for BNOH (0.003 M) and PNA (0.0036 M) in dry hexane on the silica column compare well with each other. They also compare well with the values obtained for dry hexane on the cyano bonded phase column (BNOH-0.0043 M, PNA-0.0039 M). Since CMC is a measure of a solution phenomenon, the stationary phase should have no effect and these results support that. Hernandez-Torres et al. (1986) obtained a value of 0.0035 M for dry hexane using solute retention experiments. These results also compare very favorably with other literature values as listed in Table 1.6.

There is evidence in the adsorption isotherm for the 0.1% added water system (Figure 2.5), that the CMC is shifted to higher concentrations. Surfactant loading continues to increase up to 0.02 M and then decreases as water appears to be solubilized, indicating that the CMC may be about 0.02 M. The results for the R = 10 added water system appear to confirm a higher CMC. The obtained values are 0.016 M for BNOH and 0.011 M for PNA.

The literature is conflicting with regard to the influence of water on the CMC of Aerosol OT reverse micelles. Gelade and DeSchryver (1984) state that water does not have any influence on CMC. Eicke and

Christen (1978) from theoretical considerations indicate that the CMC may be increased or decreased depending on the amount of added water.

Intuitively, since water apparently stabilizes reverse micelles, CMC would be expected to be decreased by added water. However, the large amounts added in these experiments appear to have increased the CMC and it is likely that these higher amounts of water destabilize the reverse micelles.

The results for the 3% isopropyl alcohol experiment indicate that CMC may be solute dependent in this system. It appears that solutes which partition more strongly to the micelles (RDX > PNA > methomyl), provide a lower CMC. This may be a result of solute stabilization of the micelle in a system where micellar formation is destabilized by the presence of the alcohol.

According to Equation 1.11, a plot of the inverse of capacity factor versus concentration of micellar surfactant ([AOT] - CMC) should be linear. Linearity of these plots indicates conformity with pseudophase chromatographic theory. The slope of the line divided by the intercept yields the solute binding constant per monomer. Multiplication by the aggregation number results in the binding constant per micelle.

Figure 3.8 is a 1/k versus [Mic] plot for the dry hexane mobile phase on a silica column. Table 3.2 gives the correlation coefficients obtained for the systems studied. Good correlation was obtained for linear fits in all systems, indicating that all of these systems behave as predicted by pseudophase chromatographic theory.

Also given in Table 3.2 are the micellar binding constants obtained from these plots. Aggregation numbers for the hexane and R = 10 systems were estimated using the data in Table 1.7. The R value was different at each surfactant concentration for the 0.1% water system and consequently binding constant data could not be obtained. Aggregation number data was also not available for the 3% isopropyl alcohol system. Since the alcohol should destabilize the reverse micelles, the aggregation number should be less than that for dry hexane. The hexane value was therefore used to calculate a maximum binding constant for the 3% isopropyl alcohol system.

Binding constant data for Aerosol OT reverse micelles are rare in the literature. El Seoud and Fendler (1975) have reported binding constants for imidazole, methanol and pyrazole using chloroform or deuterochloroform as solvents. The binding constants were solute concentration dependent because of self-

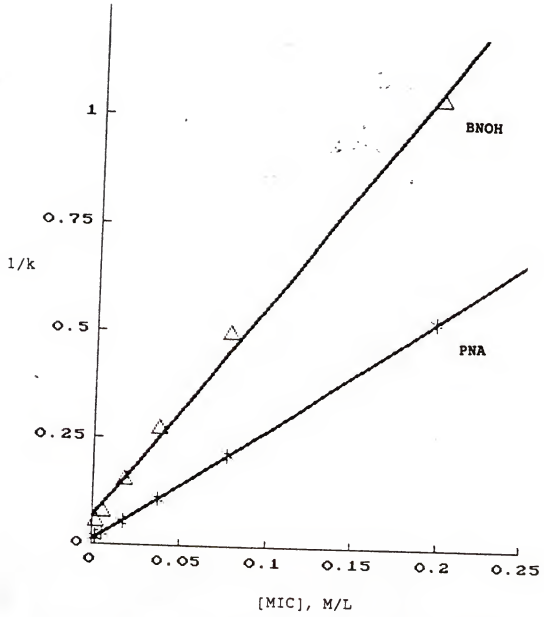


Figure 3.8 Inverse of capacity factor versus micellar surfactant concentration for a dry hexane mobile phase on an Ultrasphere silica column at 30°C.

Table 3.2 Linear correlation coefficients for theoretical pseudophase chromatography conformance in reverse micellar NPLC and derived binding constants.

SYSTEM	$\tilde{n}$	SOLUTE	r^2	BINDING CONSTANT, K_m (M^{-1})
Hexane/Silica	20	BNOH	0.996	1320
Hexane/Silica	20	PNA	1.000	4420
Hexane/Silica/R=10	137	BNOH	0.998	23500
Hexane/Silica/R=10	137	PNA	0.999	82500
Hexane/Silica/0.1%H_2O	NA	BNOH	0.996	NA
Hexane/Silica/0.1%H_2O	NA	PNA	0.998	NA
Hexane/Silica/3%IPA	<20*	PNA	0.993	<800
Hexane/Silica/3%IPA	<20*	RDX	0.970	<960
Hexane/Silica/3%IPA	<20*	MET	0.985	<150
Hexane/Cyano	20	BNOH	0.996	1540
Hexane/Cyano	20	PNA	0.996	1290

* The isopropyl alcohol should decrease aggregation number and the hexane value represents a maximum value for this system. NA = not available, IPA = isopropyl alcohol, MET = methomyl.

association of the solute molecules. This effect increased with the polarity of the solute and for methanol, the values ranged from 200 to 47 M^{-1} as the methanol concentration changed from 0.075 to 0.3 M. Pyrazole showed less concentration dependence and the values ranged from 335 to 234 M^{-1} as the pyrazole concentration was varied from 0.08 to 0.2 M.

The amount of solute used in these experiments was considerably smaller than those tested by El Seoud and Fendler (0.2 to 2 μg or 1 to 6 nanomoles injected on the column). It is anticipated that solute-solute interactions would be minimal in these experiments.

Since binding constants should be independent of the stationary phase material used, the results obtained for the silica and cyano columns should be the same. The BNOH results compare favorably, within 15%, but the results for PNA do not (4420 M^{-1} for silica, 1290 M^{-1} for cyano). Arunyanart and Cline Love (1984) have noted that intercept errors can be problematic with this technique. This appears to be what occurred for PNA on the cyano column. The slopes for the two systems compare favorably (silica - 2.61, cyano - 2.32) but the intercept for the cyano system is higher by a factor of three, causing the discrepancy.

The trends observed in these binding constants follow what would be expected. The more polar PNA

shows a higher binding constant than BNOH as does RDX compared to PNA in the isopropyl system. Water in the R = 10 system increased the binding constant of both solutes by a factor of about 20. As noted in the discussion, water creates a more polar environment in the micellar interior and also loosens the micellar structure, so increased partitioning is expected. The presence of alcohol destabilizes the micelles and also increases the polarity of the surrounding solvent. This is consistent with the observed decrease in the binding constants found in this system. The low binding of methomyl apparently arises from association of this solute with the alcohol which prevents partitioning to the micelles. This indicates a potential limitation with reverse micellar NPLC for very polar solutes and systems with polar modifiers. It may also point out a limitation with regard to loading capacity in reverse micellar NPLC systems, since solute partitioning may be concentration dependent in concentration ranges of chromatographic interest if solute-solute interactions occur in the mobile phase.

Effect of Temperature on Solute Retention in Reverse Micellar NPLC

It is also of interest to examine the effect that temperature may have on reverse micellar NPLC, since

temperature is often used as an adjustable variable in chromatography. Increasing temperature can often be desirable because it decreases retention and, as a result of lowered mobile phase viscosity, increases chromatographic efficiency and decreases operating pressures. Increased temperature can also increase the solubility of solutes in the mobile phase and can alter separation selectivity.

From simple thermodynamic considerations, it can be shown that;

$$k = \phi K \qquad \text{(eqn. 3.6)}$$

$$\Delta G^\circ = -RT\ln K \qquad \text{(eqn. 3.7)}$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \qquad \text{(eqn. 3.8)}$$

$$\ln k = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln\phi \qquad \text{(eqn. 3.9)}$$

where K is the thermodynamic distribution coefficient and ϕ is the volume phase ratio. ΔG°, ΔH°, and ΔS°, are respectively, the free energy, enthalpy and entropy of transfer of the solute from the mobile phase to the stationary phase. It follows that a plot of lnk versus 1/T, a van't Hoff plot, is predicted to be linear and

thermodynamic information about the enthalpy and entropy of the separation can be obtained from the slope and the intercept. In practice it has been found that van't Hoff plots are linear only if the basic retention mechanism does not change as the temperature is varied (Snyder, 1979; Colin et al., 1978). Dorsey et al. (1983) have examined the effect of temperature in micellar RPLC and found that linear van't Hoff plots were obtained in the range of 30 to 50°C.

Zulauf and Eicke (1979) have studied the effect of temperature on Aerosol OT reverse micelles with varying amounts of added water. As can be seen from Figure 1.5, the micellar size, and consequently the aggregation number, is independent of temperature in the range of 10 to 60°C, as long as the R (w_o) value is below about 10. With no added water, they observed a constant micellar size from -20 to 95°C. These results indicate that the reverse micelles should provide a relatively stable environment for solute partition as temperature is varied.

Experimental Two modifications were made to the basic HPLC system for these temperature experiments. A mobile phase heating coil with a volume of approximately 15 milliliters was installed in the flow stream just prior to the injection valve and was immersed in the circulator bath. Since the flow rate

was 1 ml/min, the mobile phase had 15 minutes to become equilibrated to the column temperature.

Zulauf and Eicke (1979), as well as others, have noted a phenomenon known as "boiling" for Aerosol OT reverse micellar systems. Although the boiling points of isooctane and water are 99.2 and 100°C respectively, reverse micelles with added water in isooctane "boil" at about 70°C, with a subsequent loss of water from solution. Mobile phase degassing problems, apparently associated with this phenomenon, were observed in this work at higher temperatures and a 60 psi pressure restricter was installed in the detector outlet line to eliminate the resulting signal disturbances.

van't Hoff Plots, Enthalpies of Solute Transfer, Temperature Dependence of Binding Constants

Figures 3.9 and 3.10 are van't Hoff plots for various surfactant concentrations in a 20% methylene chloride/80% hexane mobile phase on a silica column in the temperature range of 30 to 70°C. Figure 3.11 is a van't Hoff plot for 0.004 M surfactant (just above the CMC) in hexane mobile phase on an Ultrasphere cyano bonded phase column.

Table 3.3 lists the correlation coefficients for linear fits of the data for these systems. The correlation coefficients indicate an excellent linear fit of the data, confirming the integrity of the

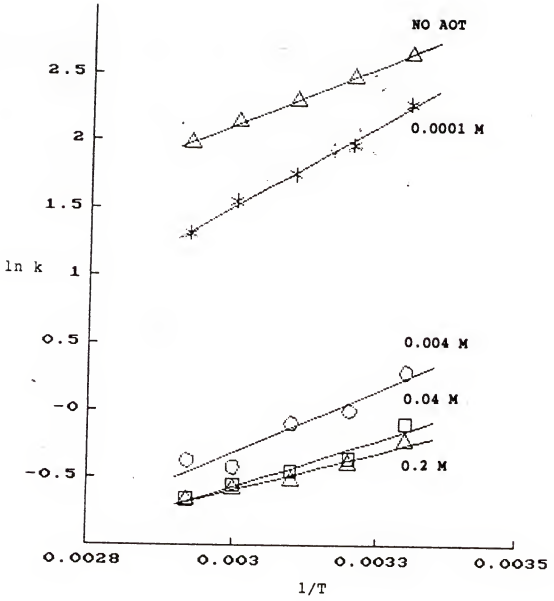


Figure 3.9 van't Hoff plots for TNT using a 20 % methylene chloride/80 % hexane mobile phase on an Ultrasphere silica column.

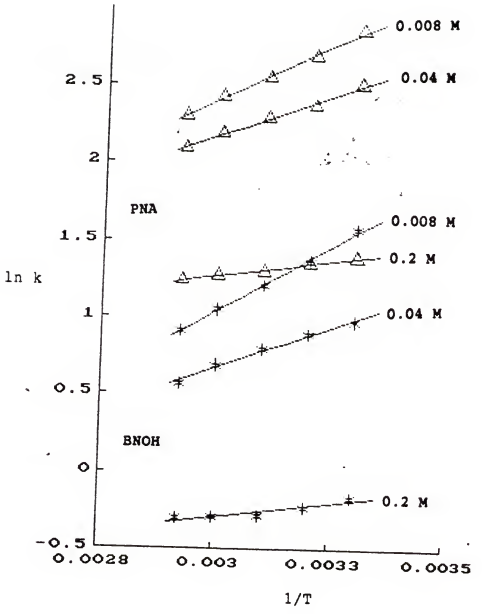


Figure 3.10 van't Hoff plots for BNOH and PNA at various surfactant concentrations using a 20 % methylene chloride/80 % hexane mobile phase on an Ultrasphere silica column.

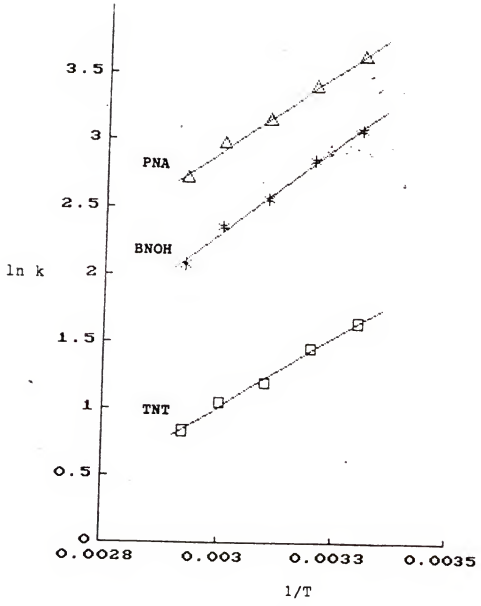


Figure 3.11 van't Hoff plots for 0.004 M Aerosol OT in hexane on an Ultrasphere cyano bonded phase column.

Table 3.3 Correlation coefficients and enthalpies for van't Hoff plots in reverse micellar NPLC.

SYSTEM	Surfactant Conc. M	Solute	r^2	ΔH°, kcal/mol
20%$MeCl_2$/80%hexane on silica	none	TNT	0.999	-8.0
"	0.0001	TNT	0.994	-11
"	0.004	TNT	0.922	-8.3
"	0.008	TNT	0.826	-7.0
"	0.04	TNT	0.957	-6.3
"	0.2	TNT	0.971	-5.1
"	0.008	BNOH	0.995	-7.9
"	0.04	BNOH	0.991	-4.8
"	0.2	BNOH	0.885	-1.6
"	0.008	PNA	0.997	-6.6
"	0.04	PNA	0.994	-4.9
"	0.2	PNA	0.993	-1.8
Hexane/cyano	0.004	TNT	0.993	-9.5
"	0.004	BNOH	0.996	-12
"	0.004	PNA	0.994	-11

reverse micelles over this temperature range. Correlation coefficients of less than 0.99 were only observed in the cases of TNT and BNOH at the highest surfactant concentrations. In these cases, the lowered correlation coefficients are a result of experimental errors involved in measuring very small capacity factors and are not due to a curvature of the data, which would indicate a change in retention mechanism.

Also listed in Table 3.3 are the enthalpy values derived from this data. As was found by Dorsey et al. (1983) in micellar RPLC, $\blacktriangle H^{\circ}$ values decrease with increasing surfactant concentration. This indicates a decreased transfer of solutes to the stationary phase as surfactant concentration increases and is due to increased partitioning of the solutes into the micellar portion of the mobile phase.

The capacity factors at the different surfactant concentrations and temperatures in the 20% methylene chloride/80% hexane mobile phase on silica system allow the estimation of binding constants at different temperatures. Figures 13.12 and 13.13 are 1/k versus [MIC] plots at the various temperatures for BNOH and PNA respectively. Table 3.4 lists the binding constants obtained from this data. The assumption was made that aggregation number did not change as a function of temperature. This assumption should be

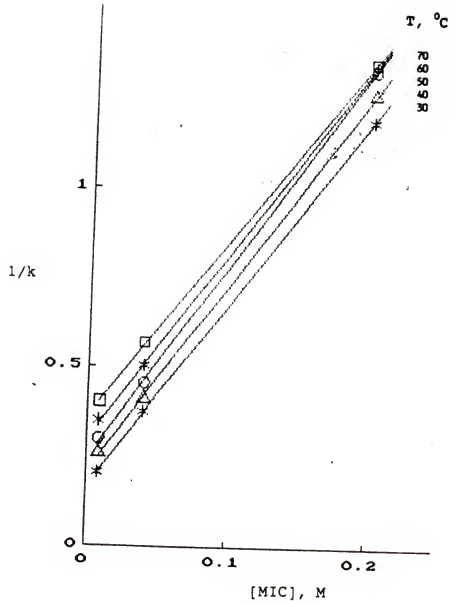


Figure 3.12 Inverse of capacity factor versus micellar surfactant concentration for BNOH in 20 % methylene chloride/80 % hexane mobile phase on an Ultrasphere silica column as a function of temperature.

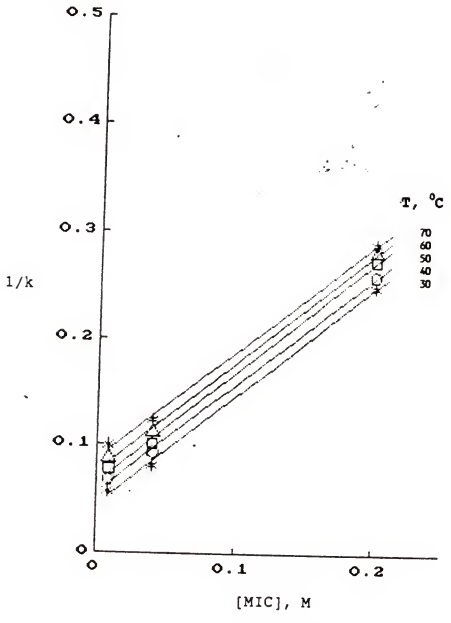


Figure 3.13 Inverse of capacity factor versus micellar surfactant concentration for PNA in a 20 % methylene chloride/80 % hexane mobile phase on an Ultrasphere silica column as a function of temperature.

Table 3.4 Micellar binding constants at different temperatures in 20% methlyene chloride/80% hexane.

Temperature, °C	BNOH* K_m (M^{-1})	PNA* K_m (M^{-1})
30	610	450
40	500	350
50	440	320
60	340	270
70	270	230

* An aggregation number of 20 was used for calculation of the binding constants.

valid given the results of Zulauf and Eicke (1979). An aggregation number of 20 was used for the calculations. Day et al. (1979) found little difference in the aggregation number between cyclohexane, toluene and chlorobenzene, therefore it seems reasonable to assume that 20% methylene chloride would change the aggregation number only slightly. The binding constant data indicate that increased temperature decreases partitioning of solutes into the reverse micelles. As far as we are aware, there is no literature available about the temperature dependence of binding constants for reverse micelles.

Conclusions

In these experiments, variables affecting solute retention in reverse micellar NPLC were examined. The reverse micellar NPLC technique was shown to be applicable to a number of systems which employed acidic and basic solutes, water and polar modifier as mobile phase components, bare silica and cyano bonded stationary phases, and temperatures between 30 and 70°C.

This technique is applicable to solutes with a wide range of polarities. However, TNT, a fairly nonpolar solute, partitioned only weakly into the reverse micelles and although solute retention exhibited some dependence on micellar concentration at

high surfactant levels, it was mostly controlled by stationary phase effects.

Conversely, methomyl, a highly polar solute, also exhibited weak partition to the reverse micelles because of solution interactions with the isopropyl alcohol modifier. Self-association of highly polar solutes at high concentrations would also be expected. These two compounds appear to delineate the two extremes of solute polarity for which reverse micellar NPLC is applicable.

Reverse micellar mobile phases provide a strong eluent for NPLC, comparable to ethyl ether at the CMC and to acetonitrile at 0.2 M. These mobile phases also have the advantage of having a linear dependence of elution strength on surfactant concentration.

The experiments performed here also allowed the extraction of physicochemical data about processes occurring in these systems. CMC's, binding constants and enthalpies of solute transfer from the mobile phase to the stationary phase were calculated, yielding information about the effects of solute, water, modifier, and temperature on them.

CHAPTER 4

CHROMATOGRAPHIC EFFICIENCY IN REVERSE MICELLAR NPLC

Introduction

Chromatographic efficiency is of fundamental importance, since the ability to resolve components of interest depends on the sharpness of the resulting elution bands. It is necessary therefore, to evaluate the effect of a change in the chromatographic technique, such as the addition of surfactant to the mobile phase, on this important parameter.

Efficiency in NPLC is dominated by effects caused by heterogeneity of the adsorption surface, in particular by the presence of a limited number of strong adsorption sites. Solutes exhibit slow desorption kinetics from these strong sites and this decreases efficiency. Generally, the more polar or ionic the solute, the more strongly it adsorbs to the strong sites resulting in increased peak asymmetry and increased band width. The addition of small amounts of water or other polar modifier is often recommended to bind these sites and thereby increase efficiency and peak symmetry (Snyder, 1968; Engelhardt, 1977; Snyder and Kirkland, 1979).

One of the drawbacks that has plagued micellar RPLC is the substantial loss in chromatographic efficiency that is inherent with the technique. The loss in efficiency originates from slow solute mass transport during the chromatographic process. In micellar liquid chromatography, there are three processes where this can potentially occur (see Figure 1.6): slow adsorption/desorption of solute on and off of the stationary phase, slow diffusion of the solute in the mobile phase and slow entrance/exit of the solute in and out of the micelles. Adsorption of solute onto the stationary phase and entrance of solute into the micelle are often considered to be fast, diffusion controlled processes (Yarmchuk et al., 1984; Almgren et al., 1979). Surfactant adsorbed on the stationary phase may cause the slow transport at the stationary phase-mobile phase interface and slow diffusion in the mobile phase is due to increased viscosity from the presence of the surfactant in solution.

The source of efficiency loss in micellar RPLC can arise from different causes depending on the system. Yarmchuk et al. (1984) found that slow exit of solute from the micelle was indicated for large solutes. Dorsey et al. (1983) showed that solute transfer between the mobile phase and the stationary phase was

slow because of poor wetting of the bonded alkyl phase by the water mobile phase and the efficiency loss may not due be to the presence of the surfactant. They showed that small amounts of propanol to aid in wetting the stationary phase and slightly elevated temperatures could provide efficiencies equivalent to those obtained using hydro-organic mobile phases.

Increased temperatures can increase solute mass transport at the micelle or stationary phase interfaces with the mobile phase, but it can also increase solute diffusion in the mobile phase. The Wilke-Chang equation is normally used for the calculation of diffusion coefficients (D_{AB}) of solutes (A) in solvents (B):

$$D_{AB} = 7.4 \times 10^{-8} \frac{(\Psi_B M_B)^{0.5} T}{\eta \tilde{V}_A^{0.6}} \quad \text{(eqn. 4.1)}$$

where Ψ_B is an association factor with a value of 1.0 for nonpolar solvents, M_B is the molecular weight of the solvent molecules, T is the absolute temperature, η is the solvent viscosity in centipoise (cp) and $\tilde{V}_A$ is the molar volume of the solute. As can be seen from equation 4.1, solute diffusion depends directly on temperature.

In turn, chromatographic efficiency has a strong dependence on solute diffusion in the mobile phase

(Snyder and Kirkland, 1979). An equation which can be written for plate height in liquid chromatography is

$$H = \frac{1}{(1/C_e d_p)+(D_{AB}/C_m d_p^2 u)} + \frac{C_d D_{AB}}{u} + \frac{C_{sm} d_p^2 u}{D_{AB}} \qquad \text{(eqn. 4.2)}$$

where C_e, C_d, C_m and C_{sm} are plate height coefficients, d_p is the particle diameter and u is the mobile phase velocity. For liquid chromatography in general, the second term of equation 4.2 (longitudinal diffusion) is insignificant at the higher velocities normally used (Karger et al., 1973) and thus plate height has an inverse dependence on solute diffusion coefficient.

Hernandez-Torres et al. (1986) have observed a substantial decrease in efficiency in reverse micellar NPLC. They found that for phenol in a 0.05 M Aerosol OT in hexane mobile phase, the efficiency was reduced from 8200 to 3300 plates on a silica column as compared to a 5% isopropyl alcohol in hexane mobile phase. The efficiency was reduced from 2400 to 900 for a surfactant mobile phase on an amino bonded phase column. The authors theorized that slow solute exit from the reverse micelles might be the cause of the efficiency loss.

Experimental Section

Apparatus The HPLC system used in these experiments has been described in Chapter 2. The

tubing used for the connections was chosen to provide minimal dead volume and band broadening. A 5 cm. piece of 1/16 inch outside diameter stainless steel tubing with 0.007 inch inside diameter (Rainin Instrument Co., Woburn, MA) was used to connect the injection valve to the column. A 15 cm. piece of the same tubing was used to connect the column outlet to the detector cell. Injection loop volume was 20 microliters. A new Ultrasphere (Beckman, San Ramon, CA) cyano 25 cm., 5 μm column was used for these efficiency experiments. Flow calibration was done using a stopwatch and a graduated test tube.

Reagents and Solutes were described in Chapters 2 and 3.

Procedure

Efficiency Measurements The efficiencies for TNT, BNOH and PNA were measured in five mobile phases at 30°C and a flow rate of 1.0 ml/min. The tested mobile phases were: hexane, 3% isopropyl alcohol in hexane, 0.0001, 0.004 and 0.2 M Aerosol OT in hexane. A minimum of five measurements was made for each solute in each mobile phase.

Foley and Dorsey (1983) have shown that the gaussian equations which are often used to calculate efficiencies in chromatography overestimate the number of plates obtained with skewed peaks. Since skewed

peaks are inherent in NPLC, an equation which was applicable to these types of bands was required for efficiency calculations in this work. Foley and Dorsey (1983) have derived an equation which allows the calculation of correct plate height values for skewed peaks:

$$N = \frac{41.7(t_r/W_{0.1})^2}{(B/A)_{0.1} + 1.25} \qquad \text{(eqn. 4.3)}$$

where A is the half width of the front of the peak and B is the half width of the peak tail, both measured at a tenth of the peak height. The width at tenth height, $W_{0.1}$, is the sum of A and B. $(B/A)_{0.1}$ is the peak asymmetry factor and gives a measure of the skewness of the chromatographic band.

Anderson and Walters (1984) have noted that the equations developed by Foley and Dorsey are only valid for peak asymmetry factors less than 2.76. They recommended the following equation

$$N = \frac{[t_r + W(0.925 - 2.17e^{-0.848(B/A)}]^2(1.06 + 54e^{-2.49(B/A)})}{W^2} \qquad \text{(eqn. 4.4)}$$

where the A, B, and W are measured at half height of the peak. In this work peak asymmetry factors greater than 2.76 were frequently observed. It was necessary to use an equation which was applicable to these peaks

and yet the data for all peak types was to be used for comparison purposes. Consequently, equation 4.4 was used for all efficiency calculations in this work. Usually the plate numbers calculated by equations 4.3 and 4.4 were not substantially different. Peak width measurements were made with a ruler and a comparator. A Lotus 123 spreadsheet program (Lotus Development Corp., Cambridge, MA) was used to perform the efficiency calculations.

Knox Plots Efficiency data was obtained at different flow velocities for 0.0001 and 0.004 M Aerosol OT in hexane mobile phases at 30°C. Reduced plate height (h) and reduced velocity (v) were calculated by the following equations:

$$h = \frac{H}{d_p} \qquad \text{(eqn. 4.5)}$$

$$v = \frac{ud_p}{D_{AB}} \qquad \text{(eqn. 4.6)}$$

$$H = \frac{L}{N} \qquad \text{(eqn. 4.7)}$$

$$u = \frac{FL}{60V_0} \qquad \text{(eqn. 4.8)}$$

where H is the plate height, d_p is the particle diameter, u is the flow velocity (cm/sec), D_{AB} is the

solute diffusion coefficient in the mobile phase, L is the column length, N is the number of theoretical plates, F is the volume flow rate and V_o is the column void volume. Diffusion coefficients for the solutes in the mobile phase were calculated using the Wilke-Chang equation (equation 4.1). Viscosity measurements showed that the viscosity of 0.0001 and 0.004 M Aerosol OT solutions were not appreciably different from pure hexane and the viscosity of hexane was used for the calculations.

Viscosity Measurements Viscosity measurements were made using an Ostwald type # 50 viscometer (Fisher Scientific, Pittsburgh, PA) at 30°C. The CRC handbook (1984c) lists the viscosity of isopropyl alcohol at 30°C as 1.77 cp. Isopropyl alcohol was used as a standard for the viscometer and the viscosities of surfactant solutions were calculated by

$$\frac{\eta_1}{\eta_2} = \frac{t_1\delta_1}{t_2\delta_2} \qquad \text{(eqn. 4.9)}$$

where η is the viscosity in centipoise, t is the time in seconds, δ is the density at 30°C and the subscripts 1 and 2 denote the reference solution and the sample solution respectively.

A value of 0.291 cp was obtained for hexane at 30°C. Interpolation of the CRC Handbook (1984) values

at 25° and 40°C gives an estimated value of the viscosity of hexane at 30°C of 0.286 cp, 1.7% different from the value obtained here. The viscosities of 0.2 M Aerosol OT in hexane and for various surfactant concentrations in the range of 0.0001 to 0.2 M Aerosol OT with added water (R = 10) in hexane were determined. Solution densities were determined at 30°C using a gravimetric procedure.

Results and Discussion

As was shown in Chapter 2, Aerosol OT surfactant exhibits strong adsorption affinity for the stationary phase materials in NPLC. Close to maximum surfactant loading is observed even at mobile phase concentrations as low as 0.0001 M surfactant. This allows the evaluation of the effect of adsorbed surfactant on chromatographic efficiency at surfactant concentrations well below the CMC. Surfactant concentration can then be increased above the CMC to evaluate the effect of the reverse micelles on efficiency.

Table 4.1 lists the theoretical plates and the asymmetry factors found for three solutes, TNT, BNOH, and PNA, on a 25 cm., 5 μm, cyano bonded phase column at 30°C. Asymmetry was high and efficiency was low, as expected, for the dry hexane mobile phase. The addition of 3 % isopropyl alcohol modifier increased efficiency and substantially decreased asymmetry for

Table 4.1 Efficiencies and asymmetry factors for reverse micellar mobile phases on a cyano bonded phase column at 30°C.

mobile phase	solute	retention time(min.)	N (plates)	asymmetry factor $(B/A)_{0.1}$
hexane	TNT	17.5	7,500	1.6
	BNOH	63.9	410	7.2
	PNA	125	140	12
3% isopropyl alcohol	TNT	9.2	8,800	1.4
	BNOH	8.7	1,100	2.3
	PNA	40.6	9,900	1.3
0.0001 M AOT	TNT	14.1	12,000	1.3
	BNOH	78.1	12,000	1.5
	PNA	149	1,400	3.2
0.004 M AOT	TNT	14.1	13,000	1.2
	BNOH	51.7	14,000	1.1
	PNA	88.2	7,400	1.4
0.2 M AOT	TNT	8.7	5,200	1.5
	BNOH	5.4	2,700	1.2
	PNA	7.1	1,500	1.4

BNOH and PNA. This is also an expected result and is due to the blocking of strong adsorption sites by the modifier. The efficiency was still low for BNOH and may be because this solute successfully competes with the alcohol modifier for the strong adsorption sites.

The results obtained for 0.0001 M Aerosol OT in hexane mobile phase indicate that the surfactant behaves in a manner analogous to the polar modifier by binding with the strong adsorption sites. The efficiencies of all solutes are greatly increased relative to the hexane mobile phase. With the exception of PNA, they are also improved compared to the 3% isopropyl alcohol mobile phase. The lower efficiency found for PNA can be attributed to it's strong retention, since an efficiency of 1400 plates is probably quite reasonable for a retention time of 149 minutes. There is no evidence of any adverse effects on chromatographic efficiency caused by the large amount of surfactant adsorbed on the stationary phase.

With a mobile phase containing 0.004 M Aerosol OT in hexane, a concentration above the CMC, 13,000, 14,000 and 7,400 plates were obtained for TNT, BNOH and PNA respectively. These efficiencies are approaching 60,000 plates/meter and are close to the best that could be obtained in NPLC, even using ideal nonpolar solutes. The peak asymmetry factors are less than 1.4

for all three solutes. In keeping with what was found in the adsorption isotherm studies in Chapter 2, these results indicate that the surfactant has tied up virtually all of the strong adsorption sites on the stationary phase. The results also indicate that slow mass transport of solute in and out of the micelle does not occur.

In order to confirm that the micelles or adsorbed surfactant were not causing slow mass transport, a study of the effect of flow velocity on efficiency was made in the 0.0001 M and 0.004 M surfactant mobile phases. The resulting Knox plots for BNOH are shown in Figure 4.1. If a slow mass transport step is incorporated into the chromatographic processes, efficiency decreases (plate height increases) rapidly as the flow velocity is increased and the resulting Knox plot rises steeply. The plots obtained for BNOH and PNA at both surfactant concentrations are relatively shallow indicating that mass transport at either the stationary phase or the micelle is not a problem in these systems.

Efficiencies are substantially decreased as the surfactant concentration is increased from 0.004 to 0.2 M and yet peak asymmetry is less than 1.5 for all solutes. The results for the 0.004 M mobile phase show no evidence of slow exit of the solute from the

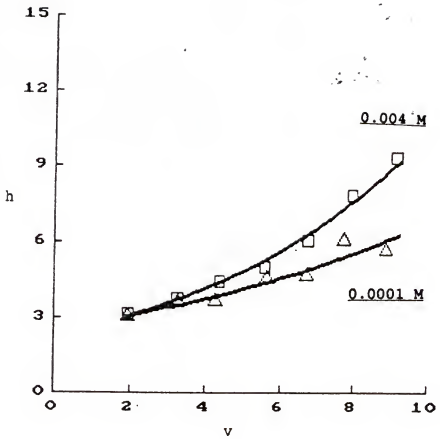


Figure 4.1 Reduced plate height versus reduced flow velocity (Knox Plot) for BNOH on an Ultrasphere cyano bonded phase column using 0.0001 and 0.004 M Aerosol OT in hexane mobile phases at 30°C.

micelle. This coupled with the low asymmetry factors, indicates that slow exit from the micelle is not the reason for the decreased efficiency at this high surfactant concentration, although it cannot be completely eliminated as a possibility. A more likely cause appears to be slow solute diffusion in the mobile phase caused by increased solution viscosity. As can be seen in Figure 4.1, the plate height for 0.004 M surfactant rises more steeply than the one for 0.0001 M and this relatively small effect may be due to the increased viscosity with additional surfactant.

As noted in the introduction, Hernandez-Torres et al. (1986) found decreased efficiency for reverse micellar NPLC. They used a relatively high surfactant concentration (0.05 M) and their results confirm those found here, that efficiency is decreased at high surfactant concentrations.

Measurements showed that the viscosity of 0.2 M Aerosol OT in hexane was 0.354 cp as compared to 0.291 cp for pure hexane at 30^0C. This would result in a 20% decrease in diffusion coefficient as calculated from equation 4.1. Figure 4.2 is a plot of solution viscosity versus surfactant concentration for Aerosol OT in hexane with added water (R = 10). Viscosity increases linearly with surfactant concentration and

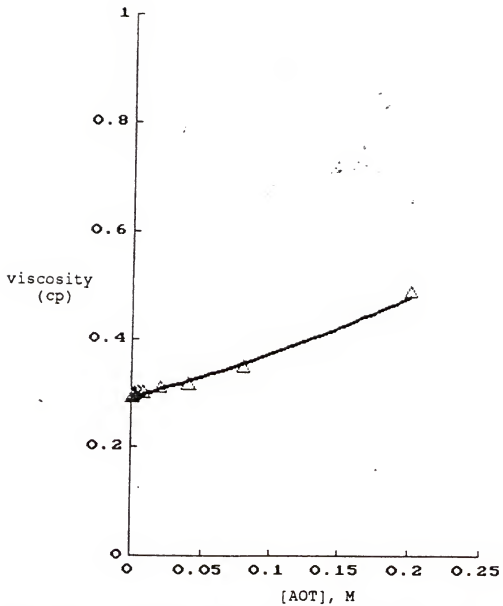


Figure 4.2 Solution viscosity versus Aerosol OT concentration in hexane with added water (R = 10) at 30°c.

thus the efficiency loss should be increased at the highest surfactant concentration.

Conclusions

The addition of small amounts of surfactant to a hexane mobile phase has a positive effect on chromatographic efficiency. The surfactant binds with strong adsorption sites, much as a polar modifier would, and results in near maximum efficiencies and low peak asymmetry. Slow mass transport at the stationary phase and reverse micellar interfaces with the mobile phase does not appear to occur.

Efficiency is decreased at high surfactant concentrations but peak asymmetry remains low. Solution viscosity increases with surfactant concentration and it appears that lowered solute diffusion in the mobile phase causes the efficiency loss. Since diffusion coefficient has a linear dependence on temperature, increased operating temperatures should be a viable means of countering efficiency loss at high surfactant concentrations. As was shown in Chapter 3, reverse micellar NPLC systems are readily amenable to increased temperatures, however the "boiling" phenomenon encountered with reverse micellar solutions may provide a practical limit to the available temperature range.

CHAPTER 5

APPLICATIONS OF REVERSE MICELLES IN NPLC--WATER MASKING

Introduction

Chromatographic solvents always contain trace levels of moisture and because dry solvents adsorb moisture from the atmosphere, the amount of water present can vary depending on the humidity of the environment where they are used. These trace levels of water can have a profound effect on the chromatography.

Due to the strong adsorption of water onto solid adsorbent stationary phase materials used in NPLC, this water is preferentially removed from the mobile phase solvent as it passes through the chromatographic column. The result is an increasing deactivation of the stationary phase with time as the mobile phase is continually passed through the column and a corresponding decrease in solute retention.

Conversely, if a very dry solvent is used in conjunction with an adsorbent that has been used previously with less dry solvents, the solvent will slowly remove water from the adsorbent causing retention to increase as a function of time. Since

solute identification is normally based upon retention time in analytical chromatography, retention time shifts present a serious problem.

In order to overcome the problem of chromatographic retention drift caused by trace levels of moisture, the addition of a large, constant amount of water (saturated or partially saturated solvents) to the mobile phase is often recommended (Snyder, 1968; Engelhardt, 1977; Saunders, 1977; Snyder and Kirkland, 1979). Although small amounts of water in the stationary phase have a large effect on solute retention, as the water content is increased above approximately 25% of saturation, the corresponding change in retention is much less. Also, in comparison to the amount of added water, the moisture which might be adsorbed from the atmosphere is negligible and thus the added water acts as a "buffer" against changes in the mobile phase water content.

In addition to eliminating retention time shifts, the use of mobile phases with added water provides several other benefits.

If the mobile phase is comprised of solvents which do not preferentially interact with specific sites on the adsorbent (i.e. alkanes and chloroalkanes), solutes are free to adsorb onto strong adsorption sites on the stationary phase. Polar solutes exhibit slow

desorption kinetics from these strong sites causing band tailing and lowered chromatographic efficiencies (Ohkuma and Hara, 1987). Also because the proportion of these high energy sites is generally small compared to the total number of sites, saturation and a non-linear isotherm can readily occur. Thus the sample loading capacity of the chromatographic system is diminished by the presence of these sites. Exceeding the loading capacity results in retention time shifts and lowered efficiency.

When water is added to the mobile phase it strongly binds onto the high energy sites effectively blocking the solute from interacting with them. Consequently the added water also serves to increase chromatographic efficiency and loading capacity.

Even though the addition of water to the mobile phase is an attractive solution to the above problems, the use of partially or totally water-saturated mobile phases can result in new difficulties. Water is a very strong mobile phase modifier and water-saturated solvents provide too strong of an eluent for many applications. Snyder and Kirkland (1979) have enumerated other problems:

a) Water-saturated solvents tend to lose water to the pores of the adsorbent leading to retention provided by a combination of adsorption and partition between the mobile phase and bulk water trapped by the stationary phase particles.

b) The amount of water taken up by the adsorbent can vary with the column's usage history.

c) When changing between water-added mobile phases, column equilibration is slow.

d) Initial equilibration for a new column is also excessively slow.

e) The preparation of water-saturated mobile phases is problematic and time consuming.

f) For the case of gradient elution with two solvents of dissimilar water solubility, water precipitation can occur on the column.

The effect of water on solute retention in NPLC has been explored by several groups of experimenters, most notably by Caude and Rosset (Szepesy et al., 1982; Souteyrand et al., 1983; Souteyrand et al., 1984). These authors determined water adsorption isotherms on alumina and silica gel out of several different solvents (di-isopropyl ether, 1,2 dichloroethane, carbon tetrachloride, chloroform and cyclohexane). These isotherms indicated that water formed a monolayer on the adsorbent which was completed at a reduced water content (water concentration in the solvent divided by the water saturation concentration in the solvent) of approximately 0.13. This portion of the adsorption isotherm could be fitted by a simple Langmuir equation. Above the region of the monolayer formation, the addition of water led to an increase in the adsorption of water onto the adsorbent which was attributed to

multilayer formation. Figure 5.1 shows an example of the experimental isotherm from Szepesy et al., 1982.

As the water content of the mobile phase is increased, the adsorption of water onto adsorption sites on the stationary phase leads to a rapid decrease in solute retention. Souteyrand et al., 1984 have shown that below the region of the completed monolayer (i.e., below reduced water contents of 0.13), there is a linear correlation between the amount of water adsorbed on the stationary phase and the decrease in solute retention.

The amount of decrease in solute retention would be expected to be solute and solvent dependent. Souteyrand et al., (1984) found some dependence on solvent and solute but the effect was not as large as might be expected. As an example, the retention (k) of five nonpolar solutes (benzene, fluorobenzene, chlorobenzene, nonadecylbenzene and naphthalene) on silica was decreased by approximately 60 to 80% as the reduced water content in cyclohexane was increased from 0.02 to 0.21 (1.8 to 20.5 parts per million, ppm). For a series of moderately polar solutes (phenol, p-cresol, 2-naphthol, aniline, p-nitroaniline, and o-nitroaniline), k was reduced by approximately 45 to

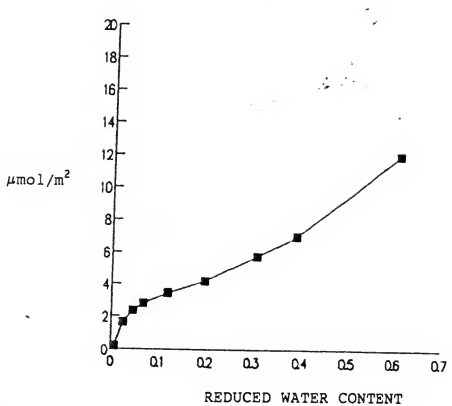


Figure 5.1 Adsorption isotherm of water on silica from chloroform. Data adapted from Szepesy et al., 1982.

65% as the reduced water contentin chloroform was increased from 0.01 to 0.27 (10.6 to 247 ppm).

For water concentrations above those required for monolayer formation, solute retention in most NPLC systems exhibits a slow decrease with increased water content. The main effect of the water is to block strong adsorption sites on the adsorbent and once these are saturated further effects are minor.

Paanakker et al., (1978) and Van den Eeckhout et al., (1987) have observed increased solute retention as the water content is increased in a few systems. Characteristically, these systems employ mobile phases that contain a large amount of a polar modifier (Snyder, 1968). In the case of Paanakker et al., (1978), n-butanol at concentrations greater than 1% or ethyl acetate at concentrations greater than 2.5% in the isooctane mobile phase and water in the range of 50 to 700 ppm were required to observe this phenomena. Van den Eeckhout et al., (1987) used mobile phases consisting of 15% methanol added to an isooctane/di-isopropyl ether mixture and containing between 0.4 and 1.4% added water. For these systems, the increase in solute retention with water content is attributed to a more favorable interaction between the solute and the multilayers of water and polar modifier adsorbed on the stationary phase (Paanakker et al., 1978; Rizzi, 1985).

As can be seen from the preceeding discussion, the presence and use of water in NPLC systems is a complicated problem and one which can have a major effect on the performance of the chromatographic system. As was discussed in Chapter 1, reverse micelles of Aerosol OT are powerful solubilizing agents for water in nonpolar solvents. Therefore, the addition of Aerosol OT to NPLC mobile phases at concentrations in excess of the CMC has the potential to eliminate problems caused by trace moisture (water masking).

The adsorption isotherm experiments (Chapter 2) and the retention experiments (Chapter 3) indicated that the Aerosol OT surfactant present in the mobile phase can shield NPLC systems from the effects of even large amounts of added water. The reverse micelles encapsulate the water in the mobile phase and although at least a monolayer of water appears to adsorb on the adsorbent surface, a monolayer of surfactant adsorbs onto the top of the water layer. This results in a very minimal change in solute retention, as compared to a dry system, even at an R value of 10. Hernandez-Torres et al. (1986) have shown that the addition of 0.05 M Aerosol OT to hexane mobile phases results in only minor solute retention shifts on amino and silica columns as amounts of water up to 1% were added.

In Chapter 4 it was shown that the surfactant provides the beneficial effects on chromatographic efficiency and peak symmetry which are normally provided by the addition of water or other polar modifier.

In the present work, water addition experiments similar to those performed by Hernandez-Torres et al. (1986) were undertaken. Of particular interest was a system in which the hexane mobile phase contained 5% added isopropanol. It has been noted several times in the literature that trace levels of moisture can affect solute retention even when using mobile phases which contain substantial amounts of polar modifiers (Paanakker et al., 1978; Rizzi, 1985; Souteyrand et al., 1984). Water masking in these types of systems would therefore be desirable. However the basic premise of the water masking technique is that reverse micelles are present to encapsulate the water and short chain alcohols tend to inhibit the formation of reverse micelles. It has been reported (Fryar and Kaufman, 1969) that as little as 1% methanol in toluene may prevent reverse micelle formation.

Experimental Section

Apparatus The HPLC pump was an Altex (Berkeley, CA) 110A equipped with a 0.45 micrometer inlet filter, a pulse dampener and a drain valve. A Rheodyne

(Cotati, CA) 7010 injection valve with a 20 microliter loop was used. A 5 micrometer, 250 x 4.6 millimeter Altex Ultrasphere cyano column was used and solute elution was monitored with an Altex Model 153 fixed wavelength (254 nanometers) ultraviolet detector with an 8 microliter flow cell. The column was maintained at 30°C by use of a glass column jacket and a Fisher Scientific Co. (Pittsburgh, PA) Model 80 constant temperature circulating bath. Data were plotted using a Scientific Products (McGaw Park, IL) variable speed chart recorder. Pump flow rate and recorder chart speed were calibrated by use of a stopwatch.

Reagents Hexane and isopropyl alcohol high purity solvents were obtained from Burdick and Jackson (Muskegon, MI) and dried using Davison (Baltimore, MD) 3A, 8-12 mesh molecular sieves. Water was obtained from Burdick and Jackson (high purity solvent). The solutes, nitrobenzene, 2,4 dinitrobenzene, and nitrobenzyl alcohol, were provided as a test mixture with the HPLC column from Altex. The Aerosol OT was obtained from Fisher Scientific Co. and used with no further purification.

Procedure Incremented water additions to the mobile phase were done in the range of 0.02 to 1.0% and the retention of the test solutes was monitored. Two mobile phases were tested; 0.05 M Aerosol OT in hexane

and 0.05 M Aerosol OT in 5% isopropyl alcohol/95% hexane. The column was an Ultrasphere cyano bonded phase thermostated at 30°C.

Mobile phases were formulated by adding volume/volume amounts of isopropyl alcohol or water to the hexane to provide the desired concentration. Aerosol OT was weighed out and dissolved in the solvent prior to water addition.

Peak retention times were measured by ruler. The void volume was taken as the point of first deviation from baseline of the solvent front.

The chromatographic capacity factor , k, was calculated from the solute retention volume (V_r) and the void volume (V_o) using the equation:

$$k = \frac{(V_r - V_o)}{V_o} \qquad \text{(eqn. 5.1)}$$

Results and Discussion

The data from the water addition experiments are listed in Table 5.1 and shown in Figures 5.2 and 5.3. The data show that essentially no decrease in solute retention occurred as a result of the addition of water with either the hexane or the 5% isopropyl alcohol

Table 5.1 Data from water addition experiments on a cyano column at 30°C.

mobile phase	added water(%)	solute retention, k NB	DNB	NBA
HEXANE	0	0.63	1.86	>26.5
HEXANE/0.05M AOT	0	0.68	1.99	7.15
HEXANE/0.05M AOT	0.02	0.68	1.95	7.07
HEXANE/0.05M AOT	0.1	0.69	2.06	7.93
HEXANE/0.05M AOT	0.25	0.72	2.24	9.47
HEXANE/0.05M AOT	1.0	0.89	2.61	11.8
5% IPA	0	0.44	1.05	2.16
5% IPA/0.05M AOT	0	0.46	1.09	2.01
5% IPA/0.05M AOT	0.02	0.45	1.08	2.07
5% IPA/0.05M AOT	0.05	0.45	1.04	1.98
5% IPA/0.05M AOT	0.1	0.46	1.11	2.27
5% IPA/0.05M AOT	0.25	0.50	1.19	2.76
5% IPA/0.05M AOT	1.0	0.69	1.66	6.38

IPA = isopropyl alcohol in hexane, NB = nitrobenzene, DNB = dinitrobenzene, NBA = nitrobenzyl alcohol.

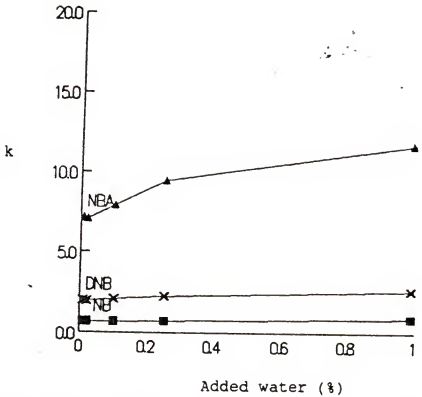


Figure 5.2 Effect of added water on solute retention for 0.05 M AOT in hexane mobile phase on a cyano column at 30°C. NB = nitrobenzene, DNB = dinitrobenzene and NBA = nitrobenzyl alcohol.

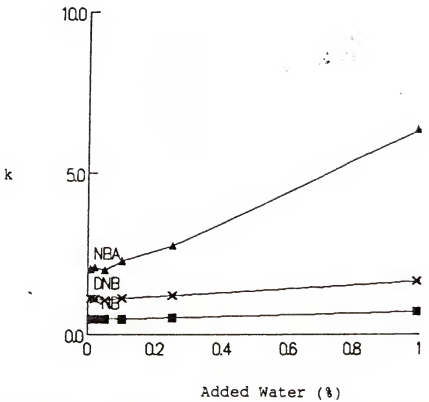


Figure 5.3 Effect of added water on solute retention for 0.05 M AOT in 5 % isopropyl alcohol/95 % hexane mobile phase on a cyano column. Solutes same as in FIgure 5.2.

mobile phases. Therefore it is concluded that the reverse micelles are effective at masking the effects of added water in NPLC, even in systems which contain 5% of a polar modifier such as isopropyl alcohol and 1% added water.

The isopropyl alcohol apparently did not prevent micelle formation in this system. As noted in Chapter 1, water is actually conducive to reverse micelle formation. Reverse micelles may not form in the absence of water and larger amounts of water promote the formation of larger micelles. It is possible that added water may counteract the tendency of the isopropyl alcohol to inhibit micelle formation.

An interesting phenomena was observed in these experiments; an increase in solute retention with the addition of water. This effect is quite pronounced for nitrobenzyl alcohol but a slight effect is also evident for the other solutes. A similar increase is also evident in the data of Hernandez-Torres et al. (1986) using a hexane mobile phase on an amino column.

Although the increased retention might be caused by the formation of a water adsorption multilayer on the stationary phase as was observed by Paanakker et al. (1978) and Van den Eeckhout et al. (1987), the adsorption experiments in Chapter 2 indicate that a surfactant layer adsorbs onto the water layer. In

addition, these multilayer effects were observed only when substantial amounts of polar modifier were present in the mobile phase. In these experiments as well as in the work of Hernandez-Torres et al. (1986), the increased solute retention was observed in mobile phases which contained no polar modifier.

Instead, it is believed that the increased retention can be attributed to a decrease in the concentration of reverse micelles as a result of the added water. As was noted in Chapter 1, micellar chromatographic theory predicts a linear dependence of solute retention on micellar concentration. Also noted in Chapter 1 and shown in Table 1.7 is the large increase in the aggregation numbers of Aerosol OT reverse micelles observed with the addition of water. Day et al. (1979) have shown that the aggregation numbers are nearly independent of the surfactant concentration (as long as it is above the CMC) and the solvent used. Consequently, the data in Table 1.7 can be used to estimate the decrease in micellar concentration resulting from the water additions in the present system. The results from using a linear fit of the data of Day et al. (1979) and the calculated R values for the water addition tests are shown in Table 5.2. They indicate that a 56% drop in micellar concentration results from the addition of 0.1%

Table 5.2 Calculated R values, aggregation numbers and micellar concentrations for water addition experiments using 0.05 M Aerosol OT in hexane as the mobile phase.

added water %	R	$\tilde{n}$*	[micelle]
0	0	11	0.0045
0.02	0.22	14	0.0035
0.05	0.56	18	0.0028
0.1	1.12	25	0.0020
0.25	2.78	46	0.0011
1.0	11.1	151	0.0003

* Aggregation numbers were calculated by fitting the data of Table 1.7 with a linear curve and calculating values from the corresponding R values.

water and that the addition of 1% water results in a 97% drop in micellar concentration.

These large decreases in the micelle concentrations would be enough to explain the increased solute retention. From the data in Table 5.1 it can be seen that nitrobenzyl alcohol is more sensitive than the other solutes to mobile phase changes in general and thus it would be expected to respond more to the changes in micellar concentrations.

The effect of the isopropyl alcohol is uncertain but it is reasonable to expect that some of the alcohol might also partition into the micelle. This would result in increasing the micellar size and aggregation number, decreasing the micellar concentration even further. In keeping with this expectation, the data in Table 5.1 indicate that the increase in retention is more pronounced with the isopropyl alcohol system. As was shown in Chapter 3, retention in a system containing isopropyl alcohol and reverse micelles is controlled by the concentration of both and the eluent strength of reverse micellar solutions is comparable to that of isopropyl alcohol mixtures.

Conclusions

The addition of reverse micelles to NPLC mobile phases appears to be an effective way to eliminate the problems caused by trace levels of moisture in mobile

phase solvents, even when the mobile phases contain substantial amounts of polar modifiers. As was shown in Chapter 4, the benefits for chromatography which result from water addition, increased chromatographic efficiency and linear capacity, are also provided by the adsorption of surfactant onto the stationary phase when using micellar mobile phases.

One problem which can occur is the decrease in micellar concentration which results when large amounts of water are added to the solvents. In practice this should not constitute a large problem, since trace levels of water do not change the micellar concentration or solute retention appreciably. The large amounts of water added in these experiments were for the purpose of emphasizing the dramatic ability of the reverse micelles to shield the chromatographic system from adverse effects.

CHAPTER 6

APPLICATIONS OF REVERSE MICELLES IN NPLC--GRADIENT ELUTION

Introduction

Gradient elution is one of the most useful techniques in liquid chromatography. In this technique, the elution strength of the mobile phase is varied during the separation and this allows the analysis of solutes of widely different polarities during the same chromatographic run. Late eluting chromatographic bands in an isocratic separation can be eluted at shorter retention times with a gradient, providing sharper peaks and improved detection sensitivity.

After the separation has been achieved in gradient elution, the chromatographic system must be returned to its initial equilibrium conditions prior to performing another analytical run. If the modifier used to adjust eluent strength is preferentially adsorbed onto the stationary phase, it must desorb during reequilibration. Often this desorption can be a slow process, especially in the case of NPLC. The time for reequilibration is added to the analytical separation

time to obtain the total analysis time and increased reequilibration time means decreased sample throughput.

In the case of micellar RPLC, surfactant monomer concentration does not increase as surfactant amounts above the CMC are added to the mobile phase, and as a result, surfactant loading onto the stationary phase remains constant. Dorsey et al. (1984) have used this property to advantage in showing that reequilibration time could be eliminated when using micellar gradients in these systems.

The adsorption isotherm studies in Chapter 2 showed that surfactant loading remained nearly constant as the mobile phase concentration was varied above the CMC for NPLC systems as well. In addition, in Chapter 3 it was shown that the reverse micelles provide a relatively strong modifier for NPLC. It was also shown that solute retention has an inverse linear dependence on surfactant concentration. These results indicate that reverse micelles may be extremely useful for gradient elution in NPLC and may require little or no reequilibration time.

Experimental Section

Apparatus The gradient system used in these studies consisted of Shimadzu Corporation (Kyoto, Japan) LC-6A pumps with a mixer controlled by a SCL-6A system controller. A Beckman (San Ramon, CA) 210 A

injection valve with a 20 μl injection valve was used for sample introduction. A Perkin-Elmer (Norwalk ,CT) LC-75 spectrophotometric detector with an 8 μl flow cell was used to monitor the eluent. The mixer and column were connected to the injection valve with 5 cm lengths of 0.007 inch id. stainless steel tubing. A 10 cm length of 0.01 inch id. stainless tubing was used to connect the column to the detector cell. Data acquisition was done using Nelson Analytical (Cupertino, Ca) model 2600 chromatography software. The column was a 25 cm long Ultrasphere with 5 μm silica packing.

Reagents The solvents, solutes and surfactant used in these experiments were described in Chapters 2 and 3.

Procedure The weak mobile phase solvent used was 0.004 M Aerosol OT in hexane, a concentration just above the CMC, and the strong mobile phase solvent was 0.2 M Aerosol OT in hexane. A major problem encountered in this work was the strong absorbance of the surfactant in the ultraviolet region which necessitated using a detection wavelength of 280 nm. Mobile phase flow rates were 1 ml/min and temperature was 30°C.

Results and Discussion

For a 0.004 M Aerosol OT in hexane mobile phase, TNT is retained about 10 minutes while PNA and BNOH are retained about 150 and 50 minutes respectively. Figure 6.1 shows a reverse micellar gradient separation of these three solutes in which PNA retention time is reduced to 27 minutes. The gradient was 10% of 0.2 M/90% of 0.004 M (0.0236 M Aerosol OT) at time 0 increased linearly to 55% of 0.2 M/45% of 0.004 M (0.1118 M) at 30 minutes. The concentration was held at 0.1118 for 2 minutes and then at 32.1 minutes was decreased to 0.0236 M over 0.1 minutes.

While the ultraviolet absorbance of the surfactant caused a baseline problem and prevented the use of detection wavelengths lower than 280 nm, it was useful for monitoring the reequilibration of the surfactant in the gradient NPLC system. The system void volume (injection valve to detector cell) was found to be 2.72 ml by measurement of the first deviation from baseline of the injection peak. The system holdup volume (mixer to detector cell) was calculated as the time difference between dropping back to initial conditions (32.1 minutes) and the time for the baseline to respond to this change (35.9 minutes) and was found to be 3.8 minutes. In an ideal system with instantaneous reequilibration, the initial conditions would be

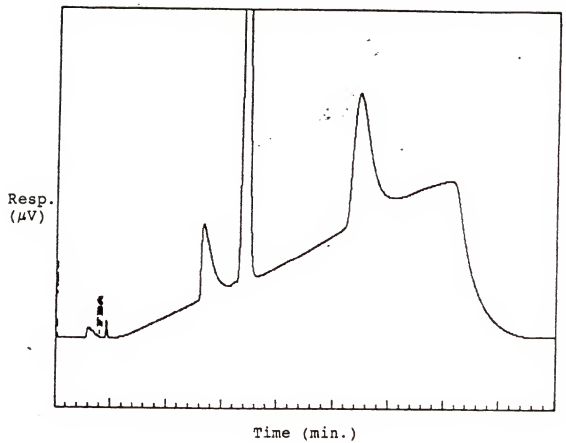


Figure 6.1 Chromatogram of a reverse micellar gradient separation using Aerosol OT in hexane mobile phase on an Ultrasphere silica column. The gradient conditions are described in the text. Solutes are: TNT-13.3 minutes, BNOH-16.8 minutes and PNA-27.3 minutes.

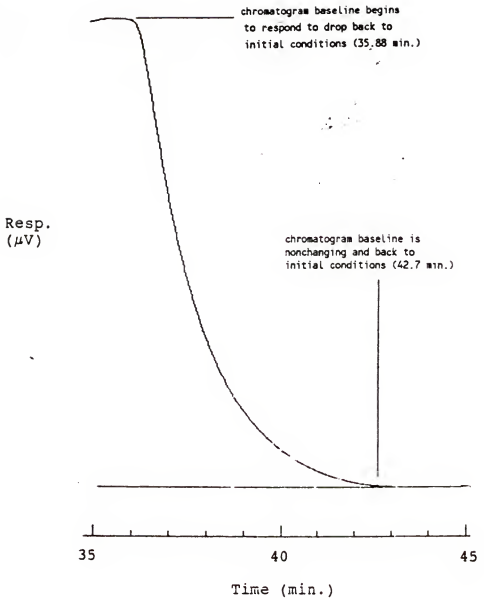


Figure 6.2 Expanded portion of reverse micellar gradient chromatogram showing the return to equilibrium conditions after the separation.

attained in the mixer at 32.2 minutes, and 3.8 minutes later, at 36.0 minutes at the detector. As shown in Figure 6.2, the time at which the system returned to a nonchanging baseline was 42.7 minutes. This indicates that approximately 7 minutes (2.5 column volumes) were required for surfactant desorption and system reequilibration. This represents a substantial reduction in the reequilibration time normally required following gradient NPLC. The silica column used in this work provides a good test of system reequilibration since bare adsorbents normally exhibit slower reequilibration than bonded phases.

Conclusions

A reverse micellar gradient in NPLC was demonstrated which provided a separation of three solutes with a wide range of polarities in a single run. The reverse micelles provide a strong modifier for use in gradient elution NPLC and eluent strength is linearly related to surfactant concentration.

The data indicate that reequilibration time may be considerably reduced by the use of reverse micellar gradients in NPLC. This is an important result because long reequilibration times are inherent in NPLC, especially when using silica or alumina.

Strong ultraviolet absorption of the Aerosol OT surfactant is a problem with this technique but the use

of other detection systems is a possibility. Other surfactants which form reverse micelles and do not absorb so strongly in the ultraviolet region, such as sodium dodecyl sulfate, may provide a solution to this problem.

CHAPTER 7

CONCLUSIONS AND FUTURE WORK

This work has focused on examining the physical processes which are operative when reverse micelles are used in NPLC. Surfactant adsorption on the stationary phase, solute partition to the micelle and micelle formation were monitored using bonded and bare adsorbents and large amounts of added water or polar modifier in the mobile phase. The results indicate strong potential for a number of applications, they also point up some limitations of the technique.

Reverse micelles can be used as a mobile phase modifier for NPLC and this work has shown that they provide a relatively strong eluent. The reverse micelles are comparable in eluent strength to acetonitrile and they should provide a unique selectivity compared to modifiers that are normally used in NPLC. As an example, the relatively nonpolar solute TNT was only slightly affected by changes in surfactant concentration while the polar solute PNA was strongly affected. This allowed both solutes to be

separated isocratically in a short time using higher surfactant concentrations.

Reverse micelles can mask the presence of even large amounts of water in NPLC. While the majority of the water is encapsulated by the reverse micelles, this work indicates that a water layer adsorbs onto the stationary phase. However, the water layer has little effect on solute retention because it is covered by an adsorbed surfactant layer. The presence of 5% isopropyl alcohol modifier did not diminish this ability. Consequently, the addition of surfactant at concentrations just above the CMC can be used to eliminate undesirable effects caused by trace moisture in a wide variety of NPLC mobile phases, even those which contain polar modifiers.

This work has pointed out several properties of reverse micellar mobile phases which are favorable for operation in a gradient elution mode. The reverse micelles provide a strong mobile phase modifier, elution strength is linearly related to surfactant concentration and surfactant loading on the stationary phase is nearly constant as surfactant concentration is varied. The last property is most important and it was shown here that very short reequilibration times were achieved when using reverse micellar gradients.

The reverse micellar NPLC technique is also useful for studying the effects of parameters such as solvent and temperature on reverse micelle formation and partition of solutes into these micelles. Binding constants and CMC's were obtained in this work for several chromatographic systems. Reverse micelle formation was shown to occur in all cases studied, when large amounts of water were present and surprisingly, even when 5% isopropyl alcohol was added to the solvent. As expected, solute binding constants increased with added water and decreased with added isopropyl alcohol and with increased temperature.

This work indicated that the retention of mildly acidic and basic solutes was not affected by their ionic character. The range of solute polarities for which this technique is applicable was delineated. Solutes which are too nonpolar (i.e. TNT) exhibit weak partition to the reverse micelles and very polar solutes (i.e. methomyl) either self-associate or associate with polar modifier which also prevents partition to the micelle. However this leaves a wide range of solute polarities for which the technique is useful.

Addition of Aerosol OT surfactant to NPLC mobile phases actually provided a beneficial effect on chromatographic efficiency and peak symmetry. This is

a result of blockage of strong adsorption sites by surfactant adsorbed on the stationary phase. Excellent efficiencies were obtained at surfactant concentrations just above the CMC. Experiments in which flow velocity was varied showed that mass transport was not slowed by adsorbed surfactant or by the micelles. Efficiency losses were observed at higher surfactant concentrations and are probably caused by increased solution viscosity. Increased operation temperature should provide an easily implemented solution to this problem and as was shown in this work, the reverse micelles maintain their integrity at temperatures as high as 70°C. Examining the effect of temperature on efficiency at high surfactant concentrations is an area for further work.

Although reverse micellar gradients appear very promising for use in NPLC, the absorption of Aerosol OT in the ultraviolet region causes a considerable baseline shift. Ultraviolet detection at wavelengths below 280 nm is not really practical with this technique. Other detector systems such as fluorescence should be feasible. The possibility also exists of using other surfactants such as SDS, which does not absorb as strongly in the ultraviolet, to form the reverse micelles. Looking at the use of SDS or other

surfactants for reverse micellar NPLC is also a promising area for further work.

The adsorption isotherm experiments indicated that the surfactant adsorbs weakly onto bonded phase sites and consequently slower reequilibration following gradients might be observed when using bonded stationary phases. Further work would also be useful in this area.

Because of the profound changes in reverse micelles caused by water addition, there exists the possibility of modifying the micelles or their selectivity using a water gradient. As an example, the size of the reverse micelles would be increased as water is added and this may provide a means for separation of macromolecules (molecules with the approximate dimensions of the micelle) according to molecular size. This may also ba an area for future work.

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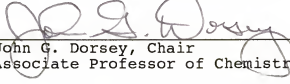
BIOGRAPHICAL SKETCH

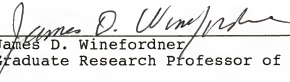
Rex Elliot Hall was born on November 18, 1952, in Alamosa, Colorado. He lived in Colorado for the first 21 years of his life. He graduated from Sierra Grande High School in Blanca, Colorado, and received a B.A. degree from Adams State College in Alamosa in spring of 1974. He moved to Florida after graduation and spent several years working in the phosphate industry near Mulberry, Florida. He also worked in the research department of a company that recovered uranium from the wet process phosphoric acid. He began attending the University of Florida in 1982 and completed his Ph.D. on a part-time basis while working full time at Environmental Science and Engineering as a analytical method development chemist. He married Jill Cora LeClair on July 3, 1982.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



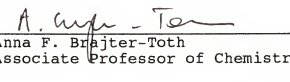
John G. Dorsey, Chair
Associate Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



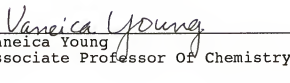
James D. Winefordner
Graduate Research Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



Anna F. Brajter-Toth
Associate Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



Vaneica Young
Associate Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Joseph J. Delfino
Professor Of Environmental Engineering Sciences

This dissertation was submitted to the Graduate Faculty of the Department of Chemistry in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1989

Dean, Graduate School